minimized samples, maximized throughput

DropSense96

User manual

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This manual supersedes all previous editions.

Contents

1. OVERVIEW 6

1.1 PRODUCT DESCRIPTION 6
1.2 INTENDED USE OF THE INSTRUMENT 7
1.3 OVERVIEW TECHNOLOGY 7
1.4 THEORY OF OPTICAL SYSTEM 8
1.5 APPLICATIONS 9
1.6 COMPONENTS 9
1.6.1 Front panel 9
1.6.2 Back panel 10

2. INSTALLATION 11

2.1 UNPACKING AND POSITIONING 11
2.2 INITIAL START-UP 12
2.2.1 Computer requirements and software installation 12
2.2.2 Software installation 12
2.2.3 Registering Your Instrument 13
2.2.4 Setting up the instrument 13

3. OPERATIONS 14

3.1 SAMPLE LOADING ON THE TRINEAN DROPPLATES 14
3.1.1 DropPlate: General description 14
3.1.2 Types of DropPlates (S and D) 15
3.1.2.1 The DropPlate-D 15
3.1.2.2 The DropPlate-S 17
3.1.3 Mode of action 18
3.1.3.1 Loading DropPlates16 on the aluminum DropFrame 18
3.1.3.2 Manual sample loading on a DropPlate 19
3.1.3.3 Automated sample loading on a DropPlate 20
3.1.4 Sample properties 20
3.2 DROPSENSE96: MODE OF ACTION 21
3.2.1 Instrument start up 21
3.2.2 Loading of a DropPlate96 on the DropSense96 21

4. DROPQUANT SOFTWARE 23

4.1.1 Start up 23
4.1.2 Main Menu 23
4.1.2.1 DropQuant Log-in 23
4.1.2.2 Main menu features 24
4.1.2.3 System info and settings 25
4.1.2.4 Measurement database 27
4.1.3 New measurement 28
4.1.4 DropPlate96 layout 30
4.1.4.1 Manual entry of sample ID 30
4.1.4.2 Import of plate layout by file (CSV, XLS and TXT import) 35

5. APPLICATIONS 40

5.1 NUCLEIC ACID QUANTIFICATION AND PURITY DETERMINATION 40
5.1.1 Theory: DNA/RNA concentration and purity determination 40

5.1.1.1 DNA/RNA concentration measurement 40
5.1.1.2 DNA/RNA purity determination 40
5.1.2 Input of nucleic acid sample data 42
5.1.3 DropPlate96 sample layout 46
5.1.4 Extra: Quantification of oligonucleotides 47
5.1.5 Extra: quantification of fluorescent labeled nucleic acids 50
5.1.6 Start measurement 52
5.1.7 Data analysis 53
5.1.7.1 96well display: 54
5.1.7.2 Column display: 55
5.1.7.3 Single well display 56
5.1.7.4 Report display 60
5.2 PROTEIN QUANTIFICATION 62
5.2.1 Theory: Protein concentration and purity determination 62
5.2.2 Starting a protein concentration measurement 63
5.2.3 DropPlate96 sample layout 68
5.2.4 Extra: Measuring multiple proteins with different extinction coefficients 68
5.2.5 Extra: quantification of fluorescent labeled proteins 71
5.2.6 Start measurement 73
5.2.7 Data analysis 74
5.2.7.1 96well display: 75
5.2.7.2 Column display: 76
5.2.7.3 Single well display 77
5.2.7.4 Report display 81
5.3 GENERAL UV-VIS SPECTROPHOTOMETRY 83
5.3.1 Introduction 83
5.3.2 Starting a UV-Vis measurement 83
5.3.3 DropPlate96 sample layout 87
5.3.4 Start measurement 88
5.3.5 Data analysis 89
5.3.5.1 96well display: 90
5.3.5.2 Column display: 91
5.3.5.3 Single well display 92
5.3.5.4 Report display 95
6. DIAGNOSTICS 97
6.1 SYSTEM INFO AND SETTINGS 97
6.2 SELF TESTING 98
6.3 POTASSIUM DICHROMATE TEST 98
6.4 QUICK SYSTEM CHECK 100
6.5 CHECK BARCODE READER (OPTIONAL ACCESSORY) 101
6.6 GENERATE STATUS ZIP FILE 102
6.7 NUCLEIC ACID CONTROLS 102

1. Overview

1.1 Product description

The DropSense96 is a unique droplet plate reader combined with microfluidic DropPlate consumables. This new generation of multichannel, polychromatic spectrophotometer and consumables is suitable for high throughput UV-VIS spectral analysis (220-750 nm) of 1-3 microliter samples in life science labs with a manual or automated workflow. The DropSense96 can also read standard 96well microtiter plates as alternative to the DropPlate consumables. The system measures the absorption properties of samples and calculates the concentration levels of the substances, e.g. nucleic acids or proteins. Using the intuitive software, the sample wells containing a specimen can be selected by a button click and the full UV/VIS absorption spectrum of the samples is displayed and stored on the connected PC.
The Trinean DropSense96 platform can be fully integrated into a automated set-up by combining it with standard liquid handling robots.

1.2 Intended use of the Instrument

The TRINEAN DropSense96 droplet spectrophotometer is intended for research and clinical use by trained personnel. The instrument is intended for the measurement of absorbance of samples in DropPlates consumables and standard 96well microtiter plates.

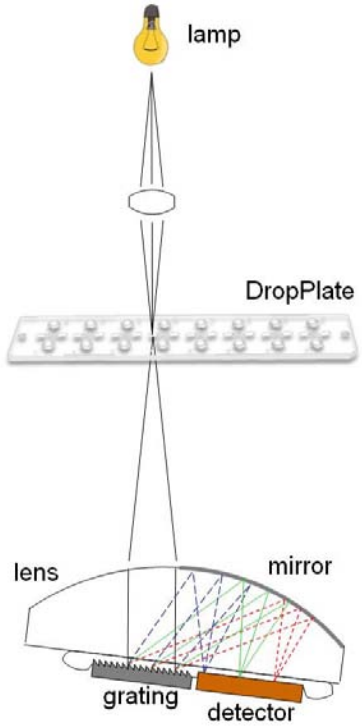
1.3 Overview technology

Using the combination of the Trinean DropSense96 with DropPlate consumables, samples are directly loaded onto the DropPlates, containing hydrophilic input wells and channels for immediate sample intake and evaporation-free storage. The following microfluidic DropPlate consumables can be used: the small DropPlate16 has 16 input wells for a low- to medium-throughput mode, and the 96well microtiter plate-sized DropPlate96 is for high-throughput use. Both are compatible with liquid handling robots (SBS standards) and permit easy loading and preservation of samples and measurement of the optical absorption of the droplet with variable path lengths. Two microfluidic alternatives are available: the DropPlate-D (Dual) has two superposed microcuvettes to perform a dual-path length measurement (0.2 and 1.0 mm) covering a large OD measurement range (0.05–110 OD, 10 mm equivalent absorbance). The DropPlate-S (Single) contains a single microcuvette with an intermediate path length of 0.5 mm, which is suitable for the high-speed analysis of samples with a more limited concentration range (0.05–44 OD, 10 mm equivalent absorbance). Alternatively, a standard 96-well microtiter plate can be used instead of the DropPlate consumables.

A pulsed xenon flash lamp provides the light source and a multichannel spectrometer with 4 linear CCD arrays in a row is used for high speed analysis of the light that passes through 4 samples simultaneous. The DropSense96 is connected to a PC for controller software, data display and additional archiving or data printing.

1.4 Theory of optical system

A functional diagram of a DropSense96 spectrophotometer module is presented here.



The DropSense96 optical core includes the following:

- A pulsed xenon flash lamp is used in the DropSense 96 to provide a wavelength range from 200 to 1000 nm.
- The sample holder, which contains the sample, allows the light to pass through and does not affect the measurement. A variety of materials are used, depending on the wavelength to be monitored. The DropPlate is commonly used as sample holders.
- The white light goes through the sample and falls upon a cylindrical lens. Light is transmitted through the lens and falls upon a grating structure that reflects the different wavelength bands to the upper part of the lens.
- Part of the lens surface has been coated with a mirror and reflects the wavelength bands back upon a highly sensitive CCD camera chip.
- Data are transmitted to the PC where data analysis software verifies and calculates the diminution in values for certain wavelengths. This enables to plot a full scan a micro- seconds of time.

1.5 Applications

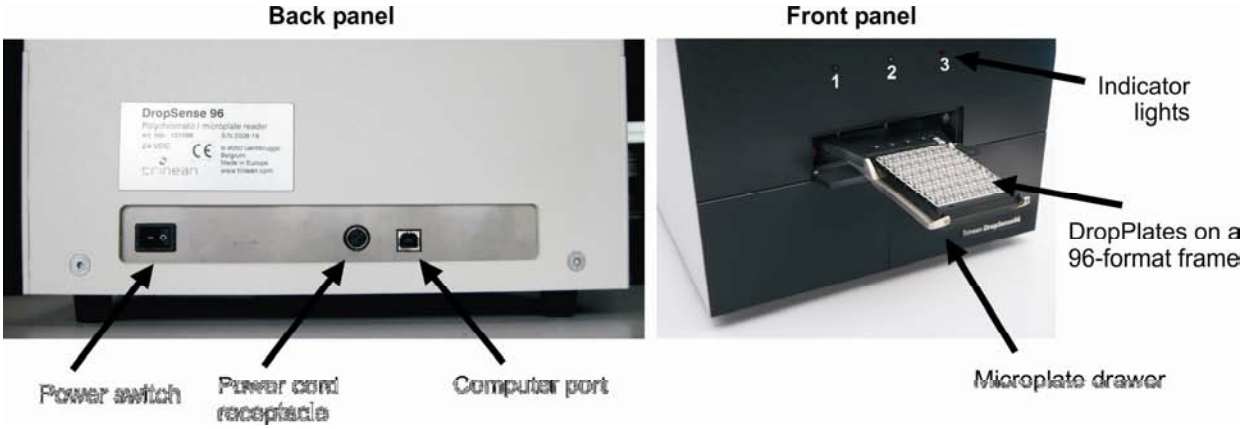
The DropSense96 couples ease of use with high speed UV/VIS spectrophotometry. The small sample requirement using the DropPlates makes the DropSense96 ideally suited for measuring:

- Concentration (A260 nm) and purity of nucleic acid samples (DNA/RNA/oligo's)
- Fluorescent dye labeling density of nucleic acid microarray samples
- Purified protein analysis (A280 nm)
- Quantification of fluorescent dye labeled proteins
- API quantification (Active Pharmaceutical Ingredient)
- General UV-Vis spectrophotometry

1.6 Components

1.6.1 Front panel

The front panel contains the central positioned microplate drawer and three indicator lights.



Indicator lights:

Three indicator lights are present: Green, Orange and Red light

For DropSense96 instruments, following light color indications are used:

1. Green, orange and red light combined: during DropSense96 start-up, all three lights will flash simultaneously for about 10 seconds
2. Green and red light: DropSense96 is ready but no communication with the PC has been achieved (check USB connection)
3. Green light: DropSense96 is ready and connected with the PC
4. Green and orange light: busy with measurement
5. Red light: error state, see "troubleshooting" or contact your distributor

Microplate drawer

The microplate drawer slides in and out of the microplate chamber that is covered by a lid on the front panel. The drawer remains in the reading chamber during measurement cycles. The drawer can accommodate: (1) DropPlates16 which are placed on a dedicated aluminum DropFrame, (2) full disposable DropPlates96 and (3) standard 96well microtiter plates. The drawer is specifically designed to be compatible with robotic handling.

The plate carrier can be opened at any given time using the 'open tray' button in the DropQuant software. Do not obstruct the movement of the tray.

1.6.2 Back panel

The following components are located on the back panel of the Dropsense96:

1. Power switch: a rocker switch, labeled I/O (for on and off, respectively)
2. Power cord receptacle: plug the power cord in here
3. Free computer USB port: Plug the accompanying USB cable into the USB port of the DropSense96, attach the other end to a USB port of the computer
4. Label: provides information about this DropSense, such as line voltage rating, cautionary information, serial number. Record the serial number shown on this label for use when contacting your supplier for technical support.

2. Installation

1. Always make sure the power switch on the instrument is in the OFF position and remove the power cord from the back of the instrument prior to any installation or relocation of the DropSense96.

2. Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and voids the instrument warranty.

3. Contact your supplier if you experience any difficulties with this instrument.

2.1 Unpacking and positioning

Remove the instrument from its packaging and inspect it for signs of damage. If any are discovered, inform your supplier immediately.

The DropSense96 is packed in a specially designed box. Please retain the box and the packing materials. If the unit should need to be returned for repair, you must use the original packing materials and carton for shipping. If the box has been damaged in transit, it is particularly important that you retain it for inspection by the carrier in case there has also been damage to the instrument.

Open the top of the box and remove the packing material from the top and sides of the instrument. Lift the instrument up and out of the shipping box. Place the instrument down carefully on a stable level surface that can take its weight (~25 kg) and position it so that air can circulate freely around the casing.

The accompanying white box contains the power cord, USB cable, the DropSense96 manual, the DropQuant installation CD-Rom and a DropPlate96 frame.

The DropSense96 is designed only for indoor use under the following conditions:

- Temperature: 10 - 35° C
- Humidity: 10-90%

If you use the instrument in a room subjected to extremes of temperature change during the day or before use stored in a cold environment, it should be allowed to come to thermal equilibrium for 2-3 hours in the laboratory before switching on. This will prevent calibration or self-test (upon start-up) failure as a result of internal condensation.

Make sure that no DropPlate disposables are located inside the DropSense96 instrument during transportation. This may cause damage to the internal mechanics of the DropSense96.

2.2 Initial start-up

2.2.1 Computer requirements and software installation

The operating software will only run on a PC meeting the below criteria. No Mac versions of the software are currently available.

- Microsoft Windows XP, Vista or Windows7 operating system
- Microsoft .NET Framework version 2.0 or higher
- 1.5GHz or higher processor
- CD ROM drive
- 1GB or more of RAM (2GB if running Vista)
- 100 MB of free hard disk space for software installation
- Free USB port (the instrument can only be connected via the USB port)
- Microsoft Excel and Adobe pdf reader to manipulate archived data (optional)

The system software must be loaded onto the PC before the USB cable is connected. Administrator access on the PC is required to install the software.
When attaching the USB cable, please wait at least 30 seconds for the USB devices and internal drivers to be installed and recognized.

2.2.2 Software installation

To properly install the operating software:

- Close all programs and make sure that the USB cable is unplugged.
- Insert the operating software CD in the CD drive of the PC. The software installation menu should appear automatically. If software menu does not appear, choose 'My Computer' to view the contents of the CD. Double click on the file named 'setup.exe'.
- The first screen will ask the installation path. It is recommended to use the default installation path.
- After accepting the License Agreement, the DropQuant software will be installed
- Finally, some additional installations will be performed. These installations are necessary if you want to make full use of the DropQuant software.
    - PDF Creator is used to create PDF reports
    - SQLite is used to create a database of past experiments
    - USB driver is necessary to make connection to the DropSense instrument

If these additional installations were not performed during initial software installation, then they can be installed afterwards from Start > All Programs > Trinean DropQuant > Additional installers

- Before you start the DropQuant software, power on the instrument and connect the USB cable.
- Start the DropQuant software by the shortcut on the desktop, or go to

  Start > All Programs > Trinean DropQuant > DropQuant

2.2.3 Registering Your Instrument

Please register your instrument! We periodically update our software and add new features free of charge. We keep our user list updated so that we can alert you to these updates. All information supplied is completely confidential.

Please register your instrument on our website (www.trinean.com/user/register) or mail to info@trinean.com

2.2.4 Setting up the instrument

Install your DropSense96 instrument as follows:

- Place the DropSense96 on a stable, level surface that can take its weight (~ 25 kg) away from direct sunlight, dust, drafts, vibration, and moisture. Position it so that air can circulate freely around the casing.
- Turn the DropSense96 around so that the back is facing you and insert the USB cable into the USB port receptacle. Attach the other end to the computer. Connect the instrument to the power supply with the power adaptor supplied.
- Turn on the power to start the instrument (rocker switch at the back). Start-up the software so that a connection can be established.

Caution! Strong light-source! Never look directly into the beam of any UV/Visible spectrophotometer.

The instrument is fitted with safety interlocks. If the instrument is used in a manner not specified or in environmental conditions not appropriate for safe operation, the protection provided may be impaired and instrument warranty withdrawn. There are no user-serviceable parts inside this instrument.

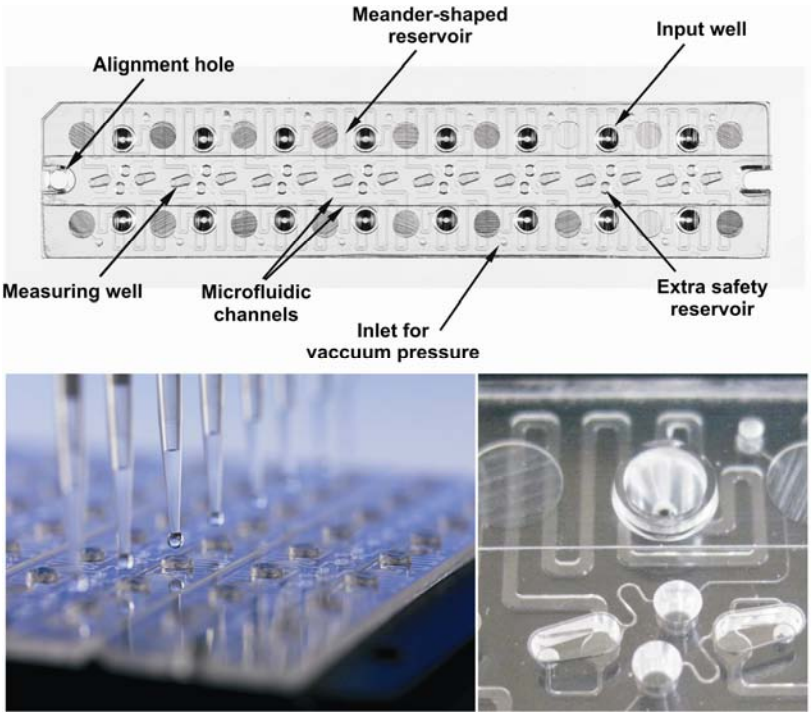
3. Operations

3.1 Sample loading on the Trinean DropPlates

3.1.1 DropPlate: General description

The Trinean DropPlate consumables have been designed for specific use with the DropSense96 and are made of special optical quality plastic allowing transmission of UV-VIS spectra. The conical-shaped input wells are positioned with a 9mm (SBS standard) pitch fit for multi-channel pipettors or robotic sample loading. These input wells are connected with a capillary storage channel and one or two micro-cuvettes, as shown in the figure below. The microfluidic structures continue with a small channel leading to a small upper vent used as the inlet for the vacuum pressure system. When dispensing a sample droplet into the input well, it is instantly drawn into the storage channel through capillary forces in order to strongly suppress sample evaporation. This allows the user to perform the measurement on the DropSense96 within a time span of 2h after dispensing.

After inserting the DropPlate into the DropSense96, pressure-driven transport of the samples to the micro-cuvettes occurs while simultaneous absorbance measurements are performed to monitor the filling behavior of the micro-cuvettes and analyze the spectral absorbance of the sample. The microfluidic chip provides steady measuring conditions due to fixed path lengths and elimination of solvent evaporation, leading to enhanced reproducibility.



Two DropPlate formats can be read on the DropSense96 spectrophotometer:

- **DropPlate16**: this small version of the DropPlate consumable has 16 input wells for a low- to medium-throughput mode. For low sample numbers, 1 to 6 DropPlates can be assembled on a aluminum DropFrame creating a flexible set-up measuring 1 to 96 samples simultaneously. For higher sample numbers, assembly of 6 DropPlates on a frame will generate a 96well microfluidic plate with identical outer dimensions as a standard 96well plate, assuring compatibility with liquid handling robots.
- **DropPlate96**: the 96well Microtiter plate-sized DropPlate96 is suited for high-throughput use. This consumable is ideal for automated workflows as it is easy stackable and bar-coded for sample tracking. The DropPlate96 is compatible with liquid handling robots (SBS standards) and permits easy loading and preservation of samples and measurement of the optical absorption of the droplet.

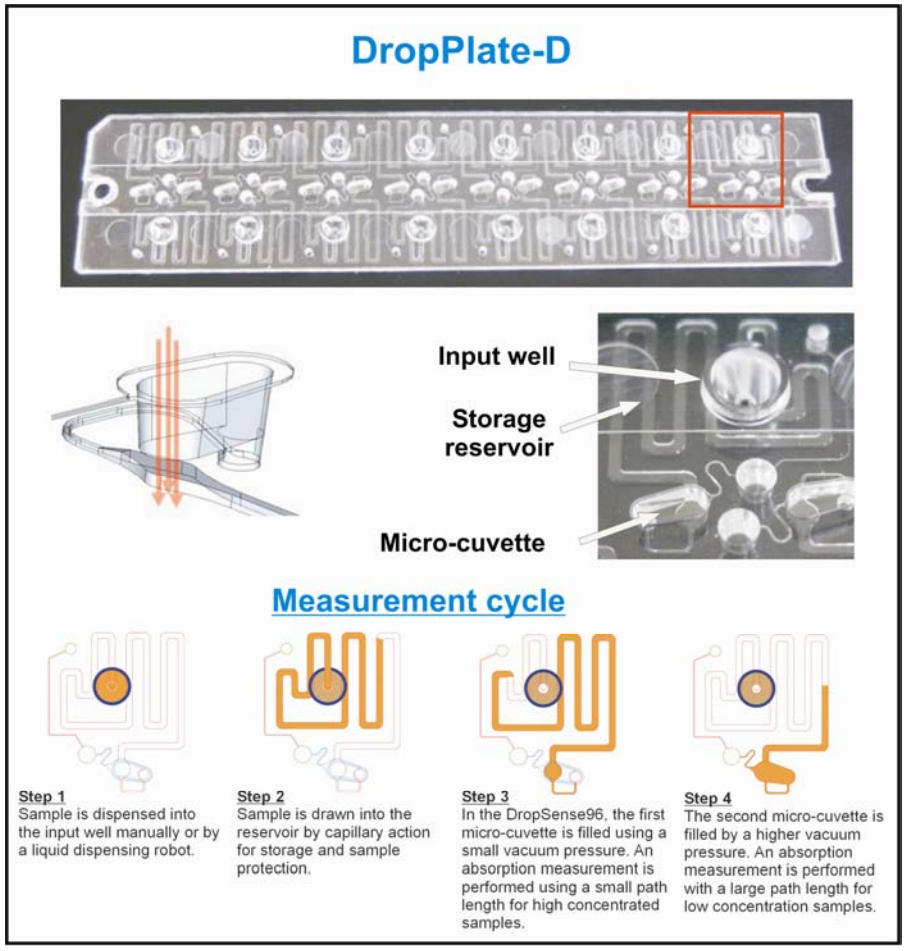
Note: It is not necessary to fill all 16 input wells to perform a measurement. A single measurement can be done and unused positions on a DropPlates can be filled in another measurement until all input reservoirs have been filled.

3.1.2 Types of DropPlates (S and D)

Two DropPlate versions have been developed with slightly different microfluidic structures: the DropPlate-S (Single) and DropPlate-D (Dual). Both have identical conical-shaped input wells connected with a meander-shaped storage reservoir that uses capillary force to take up the sample from the input well and protect the small samples from evaporation. The other end of the meander reservoir is connected to one (DropPlate-S) or two micro cuvettes (DropPlate-D) for UV-VIS analysis. The choice of DropPlate16 to be used depends on the desired OD measurement range of the experiment and measurement speed, as explained below.

3.1.2.1 The DropPlate-D

The DropPlate-D (Dual) contains a meander-shaped reservoir that can store up to 3.5 µl of sample and two superposed micro-cuvettes for optical analysis. By consecutive filling of both optical chambers, a dual path length measurement is performed (0.2mm and 1.0mm). The combination of both path lengths results in a large OD measurement range and thereby omits the need for sample dilution.

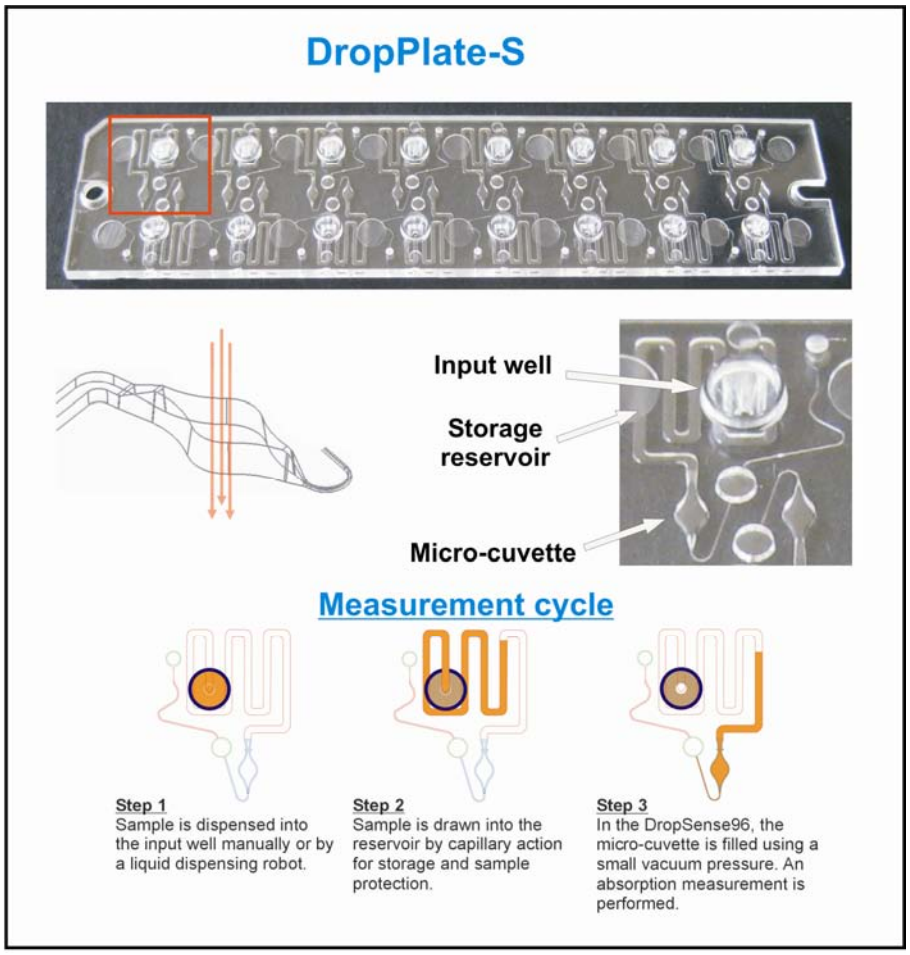

DropPlate-D
Input well
Storage reservoir
Micro-cuvette
Measurement cycle
Step 1
Sample is dispensed into the input well manually or by a liquid dispensing robot.
Step 2
Sample is drawn into the reservoir by capillary action for storage and sample protection.
Step 3
In the DropSense96, the first micro-cuvette is filled using a small vacuum pressure. An absorption measurement is performed using a small path length for high concentrated samples.
Step 4
The second micro-cuvette is filled by a higher vacuum pressure. An absorption measurement is performed with a large path length for low concentration samples.

Depending on the required OD-range, a choice can be made for a single or dual chamber measurement. The single chamber measurement has a path length of 0.2mm with an OD range of 1-75 OD (for a 10mm equivalent path). This single chamber mode is ideally suited for highly concentrated samples. The dual chamber measurement option contains a dual path length (0.2 and 1mm) measurement, creating an OD range of 0.05-110 OD (for a 10mm equivalent path). The dual path length option has a wider measurement range then the single chamber mode and is suited for the majority of biomolecule samples. A selection for the single or dual chamber measurement can be done in the DropQuant software.

Sample size requirements: Although the dispensed sample volume is not critical, it is essential that a minimal amount of sample is dispensed for correct filling of the measurement chambers allowing precise measurements. Extensive testing indicates that sample volumes of 1.5 µl for a single chamber measurement and 3 µl for a dual chamber measurement are sufficient to ensure reproducibility. Although this volume range takes a pipetting error into account, it is best to use a precision pipettor (0.5-5 µl) with precision tips to ensure that sufficient sample is used.

3.1.2.2 The DropPlate-S

The DropPlate-S looks very similar to the DropPlate16-D, however the meander reservoir is shorter and can include up to 2.5 µl samples. Furthermore, the DropPlate16-S contains only one micro-cuvette with a path length of 0.5mm. The 0.05-44 OD range (for a 10mm equivalent path) of the DropPlate-S is ideally suited for quantification of biomolecule samples derived from automated extraction procedures with a stable yield. For example, extracted human genomic DNA tends to be within a 50-1000 ng/µl concentration range and can be quantified using the DropPlate-S. Since the DropPlate-S contains only one micro-cuvette instead of two like the DropPlate16 D, the measuring time for a full 96well DropPlate-S is decreased by halve to about 4 minutes.


DropPlate-S
Input well
Storage reservoir
Micro-cuvette
Measurement cycle
Step 1
Sample is dispensed into the input well manually or by a liquid dispensing robot.
Step 2
Sample is drawn into the reservoir by capillary action for storage and sample protection.
Step 3
In the DropSense96, the micro-cuvette is filled using a small vacuum pressure. An absorption measurement is performed.

Sample size requirements: Although the dispensed sample volume is not critical, it is essential that a minimal amount of sample is dispensed for correct filling of the measurement chamber allowing precise measurements. Extensive testing indicates that sample volumes of 2 µl is sufficient to ensure reproducibility. Although this volume range takes a pipetting error into

account, it is best to use a precision pipettor (0.5-5 µl) with precision tips to ensure that sufficient sample is used.

Overview of DropPlate types

	DropPlate 16	DropPlate96
DropPlate-S	16 input wells + aluminum DropFrame 2 µl samples OD range: 0.1-44 OD dsDNA: 5 – 2200 ng/µl IgG: 0.15 – 33 mg/ml	96 input wells Full disposable 2 µl samples OD range: 0.1-44 OD dsDNA: 5 – 2200 ng/µl IgG: 0.15 – 33 mg/ml
DropPlate-D	16 input wells + aluminum DropFrame 3 µl samples OD range: 0.1-110 OD dsDNA: 5 – 5500 ng/µl IgG: 0.15 – 95 mg/ml	96 input wells Full disposable 3 µl samples OD range: 0.1-110 OD dsDNA: 5 – 5500 ng/µl IgG: 0.15 – 95 mg/ml

3.1.3 Mode of action

3.1.3.1 Loading DropPlates16 on the aluminum DropFrame

Up to 6 DropPlates16 can be placed on a DropPlate frame, creating a full 96well plate with standard 96well plate dimensions (SBS standard). The DropPlates16 can only be placed in one way on the DropFrame due to the alignment pins and orientation triangles on the frame.

It is recommended to put the DropPlates16 on the frame before loading the liquid samples. The DropFrame has been made black to aid the visual inspection of loaded or empty input wells and associated sample reservoirs. This specific design of the DropFrame minimizes human errors during sample loading.

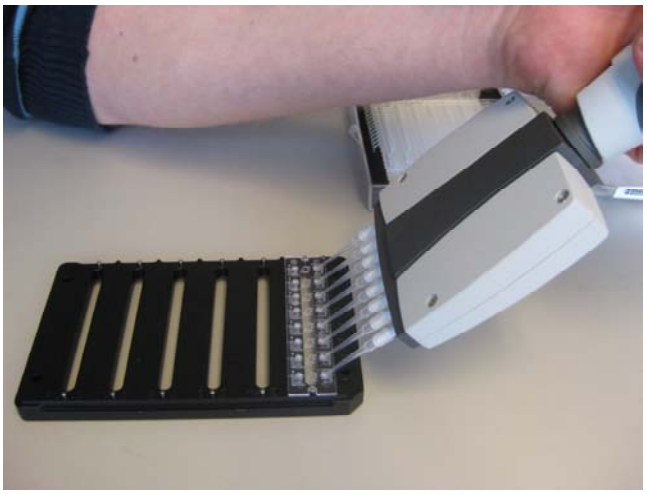
Stackable DropPlate 96 frames
Alignment pins for DropPlate16 positioning
Orientation triangle on the DropPlate96 frame

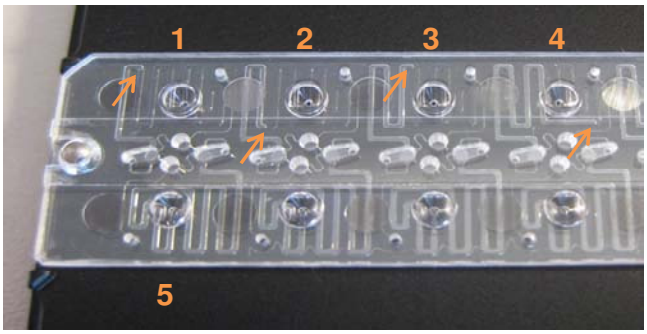
3.1.3.2 Manual sample loading on a DropPlate

Take care not to introduce large air-bubbles into the dispensed sample. When dispensing samples into the DropPlate, put the tip of the pipettor into the input well at an angle of 10° angle (or more) until the tip makes contact with the inner wall of the well and then deliver the liquid by gently pressing the push button of the pipettor until the first stop (pressing further down to the second stop inevitably will create bubbles in the sample). Lift the pipette up carefully after dispensing. The sample will be sucked automatically into the meander reservoir by capillary force.

The black colored aluminum DropFrame and 96well mould of the DropPlate96 create a difference in visual contrast between empty and filled meander-shaped reservoirs of the DropPlate disposables. This allows a quick and easy visual control of the loaded wells. Furthermore, this visual inspection can be used to estimate the amount of sample dispensed or the presents of air bubbles in the samples, as shown in the figure below.

The dispensed sample will automatically move into the channel reservoir by capillary force. Therefore, the sample does not need to be pushed into the reservoir by the pipettor.





DropPlate-D
1: 3 µl
2: 2.5 µl
3: 2 µl
4: 1.5 µl
5: empty

3.1.3.3 Automated sample loading on a DropPlate

Liquid handling robots with 1 to 96 pipetting heads can be used for dispensing samples in the DropPlates. The pipetting head must approach the DropPlates vertically and the distance between the head and the input wells should be optimized to ensure that the dispensed sample can make contact with the inner wall of the input well (the pipetting head shouldn't make contact with the input wells). The conical shape of the input well and its hydrophilic nature will guide the droplet deeper in the input well to ensure that the sample is drawn into the anti-evaporation reservoir.

For additional information please contact info@trinean.com

3.1.4 Sample properties

Sample carryover is prevented by the DropPlate design: the elevated edges of the input wells avoid sample carryover within a DropPlate and the extra safety features on the DropPlate avoid contamination of the pressure manifold of the DropSense96.

Sample homogeneity needs to be ensured for measurement precision. Sampling from non-homogeneous solutions can cause significant deviations in the data generated using small volume spectrophotometers. Highly concentrated nucleic acid samples and other viscous solutions are common examples known to the molecular biologist.

Due to the small volume requirements by the DropSense96, it is important to ensure that the sample being measured is homogeneous and not too viscous. This is important for correct pipetting and avoids measurement deviation in the data generated.

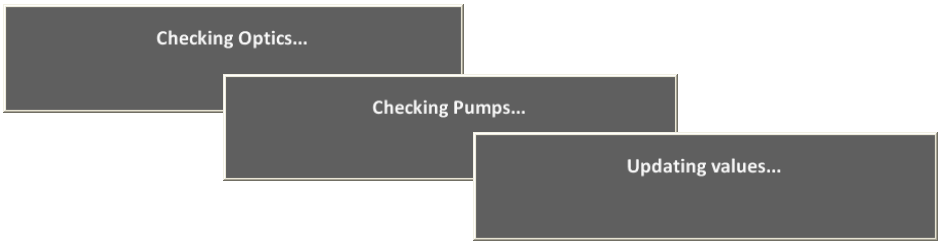
Genomic DNA, lambda DNA and viscous solutions of highly concentrated nucleic acids are common examples that require careful attention to ensure homogeneity before sampling. Furthermore, proteins can be subject to denaturation, precipitation, and aggregation and therefore may require special handling to ensure sample homogeneity.

Sample evaporation is minimized due to the meander-shaped sample reservoir that takes up the sample automatically during sample dispensing. This design avoids large measurement inaccuracy due to evaporation effects and sample concentrations remain constant for about 2 hours.

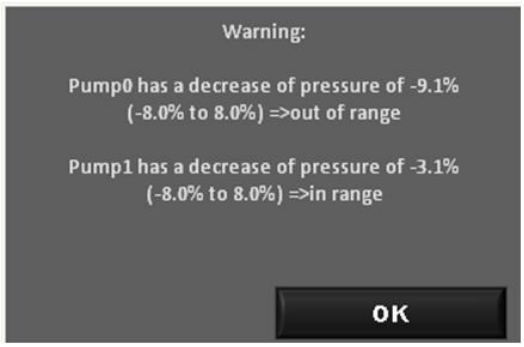
3.2 DropSense96: Mode of action

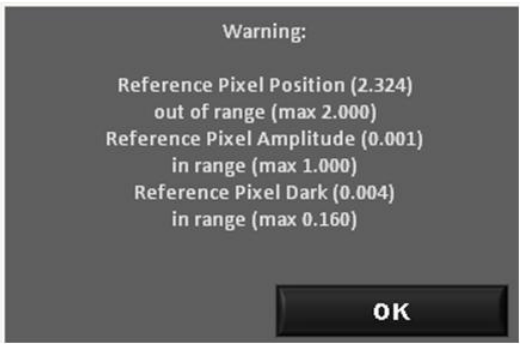
3.2.1 Instrument start up

The power switch is located on the back panel of the DropSense96. Push the rocker switch to the ON position. The instrument automatically pulls in the microplate tray when the tray was in the open position. When the DropSense96 instrument is powered on, double-click the DropQuant software icon on the computer desktop to start the program. Once logged in, a first message will indicate that the USB connection is being made, the instrumental hardware settings are being read and diagnostic checks are performed to ensure correct functioning.



If the pumps or spectrophotometer don't perform as expected during the diagnostic check-up, an information window will automatically pop-up (see examples below). Please contact your distributor for a thorough system check and recalibration.

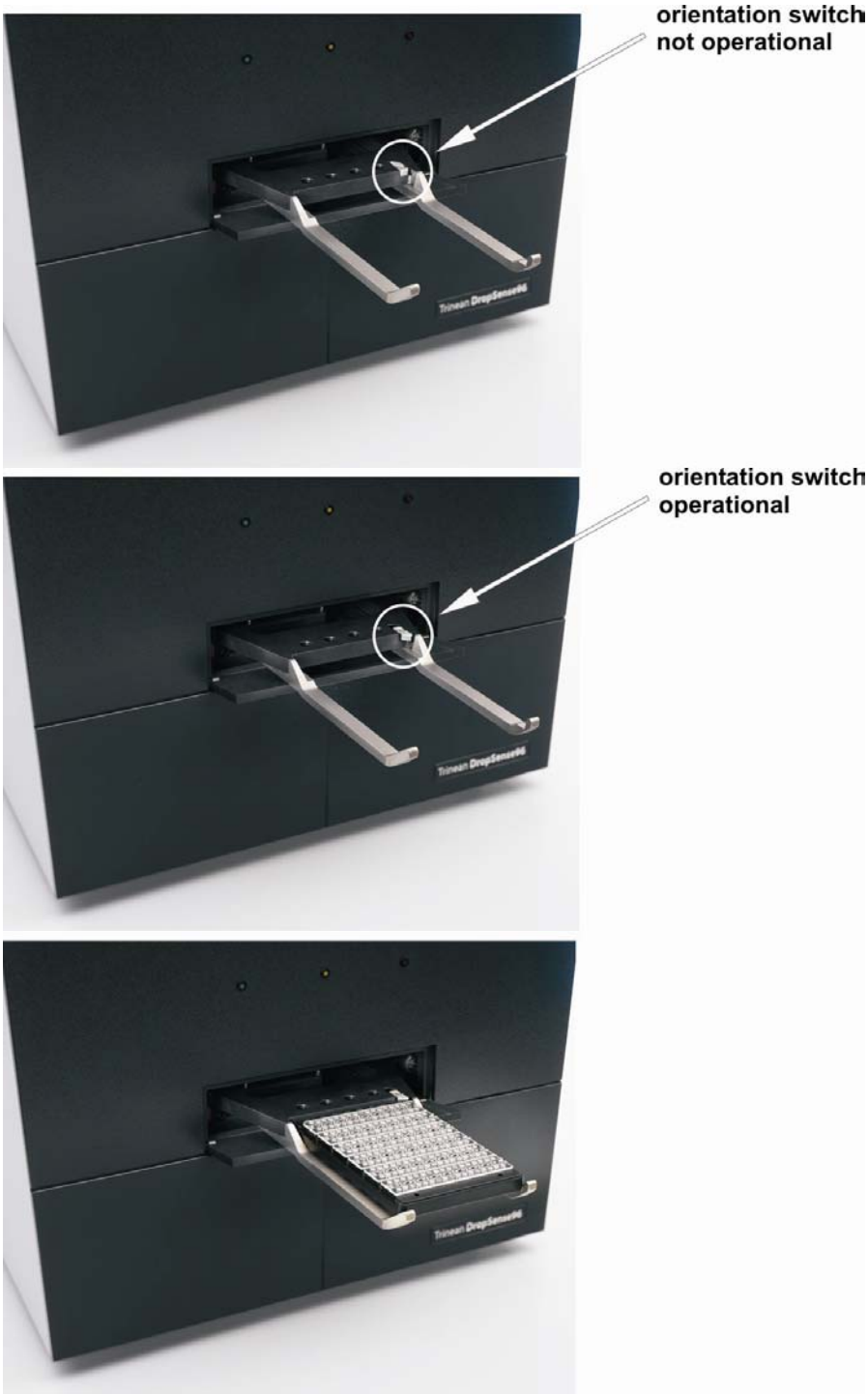

Warning:
Pump0 has a decrease of pressure of -9.1%
(-8.0% to 8.0%) =>out of range
Pump1 has a decrease of pressure of -3.1%
(-8.0% to 8.0%) =>in range
OK



3.2.2 Loading of a DropPlate96 on the DropSense96

The plate carrier of the DropSense96 can be opened at any time (except during measurements) using the 'open/close tray' button in the DropQuant software. The tray opens automatically when pushing the 'start test' button in the software (see further) to load the DropPlate96 or a standard 96well microtiter plate before reading. When reading is complete, the drawer of the DropSense96 opens, allowing you to remove the DropPlate. The drawer closes automatically after 30 seconds (this feature is not active when the DropSense96 is integrated).

The DropPlate must be loaded onto the microplate drawer of the DropSense96 with the well A1 matching the upper left position of the drawer. An orientation switch in the upper left corner can be used to avoid incorrect placement of the frame on the drawer. Make sure that the DropPlate96 frame is placed accurately and flat on the drawer.



4. DropQuant software

4.1.1 Start up

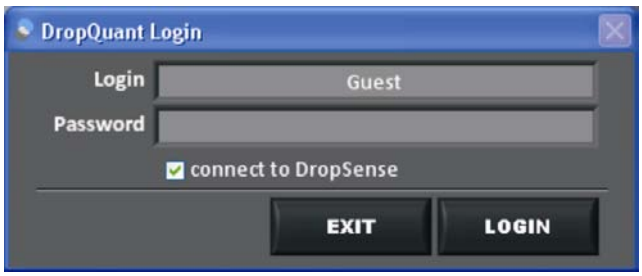
To ensure that the DropQuant software can communicate with the DropSense96 instrument, connect the instrument to the computer and turn on the DropSense96 prior to starting the software.

Start the operating software by selecting the following path: Start ▸ Programs ▸ DropSense 96 or by a double mouse-click on the DropQuant software icon on the desktop.

4.1.2 Main Menu

The software opens to display the main menu.

4.1.2.1 DropQuant Log-in



This screen is used to:

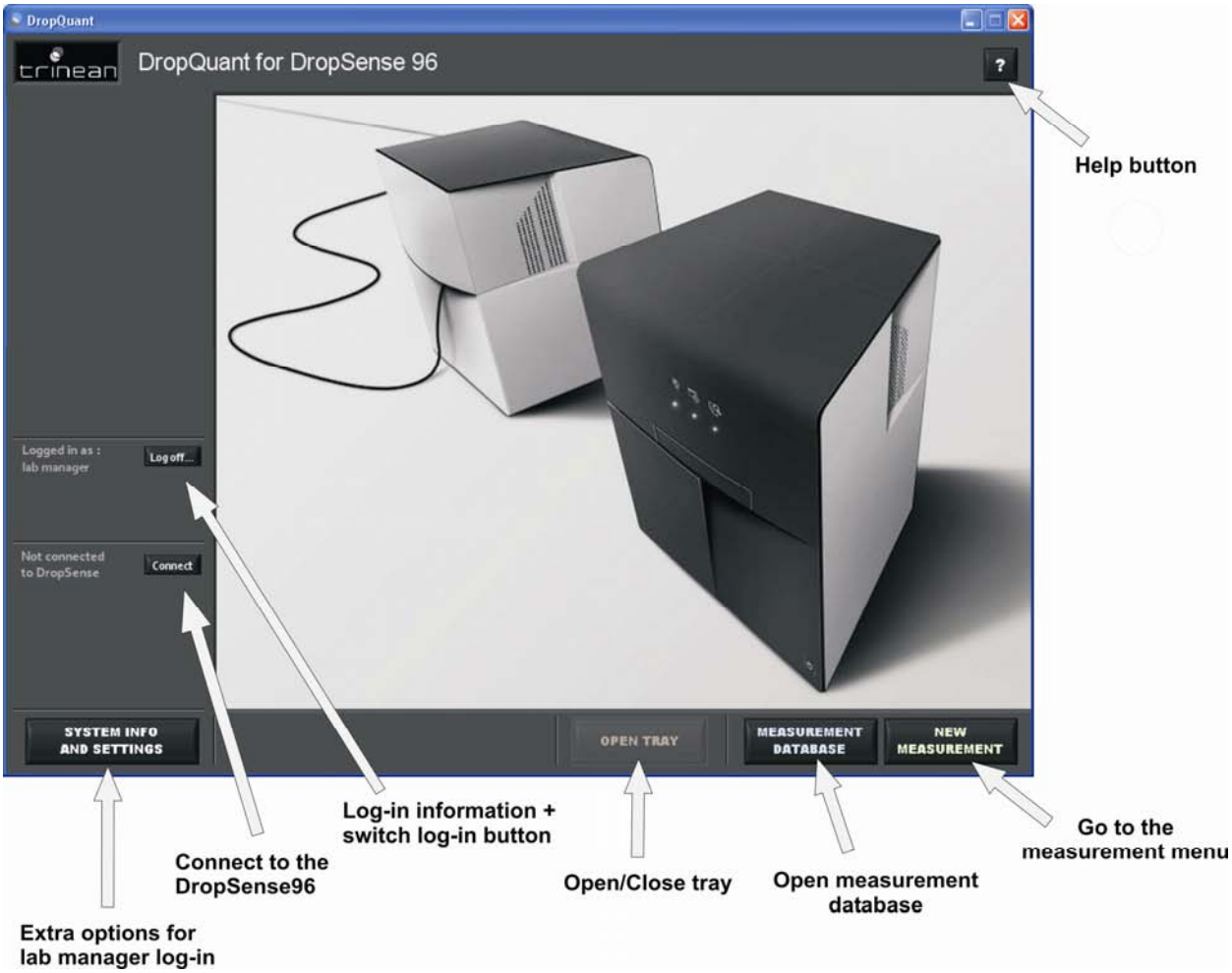
- Change the user name/account.
- Exit the DropSense96 software
- Choose to connect the software with the DropSense96 or use the software for data analysis without connecting to the instrument

Pre-defined accounts are:

- **Lab manager.** This account has full access to the software and has the possibility of changing the number of accounts, their passwords and the location of the measurement database. It is recommended to change the passwords of the different accounts when installing the DropSense96.

The 'lab manager' log-in is protected by a predefined password. This password differs on every DropQuant installation CD and the correct password can be found on the DropQuant CD included in the installation box.

- **User1**, no predefined password. Second level, can use the full operational software.
- **Guest**, no password needed. Limited access to the software.



4.1.2.2 Main menu features

Log off…

Use this button to change the account or to close the DropQuant software

Connect

This button is only shown when the software is not connected to a DropSense96. When connection is needed, plug the DropSense USB cable in, and press 'connect'.

Open/Close tray

With this button, the plate carrier can be opened or closed at any moment.

Help Button

This button opens the user manual in pdf form

System info and settings

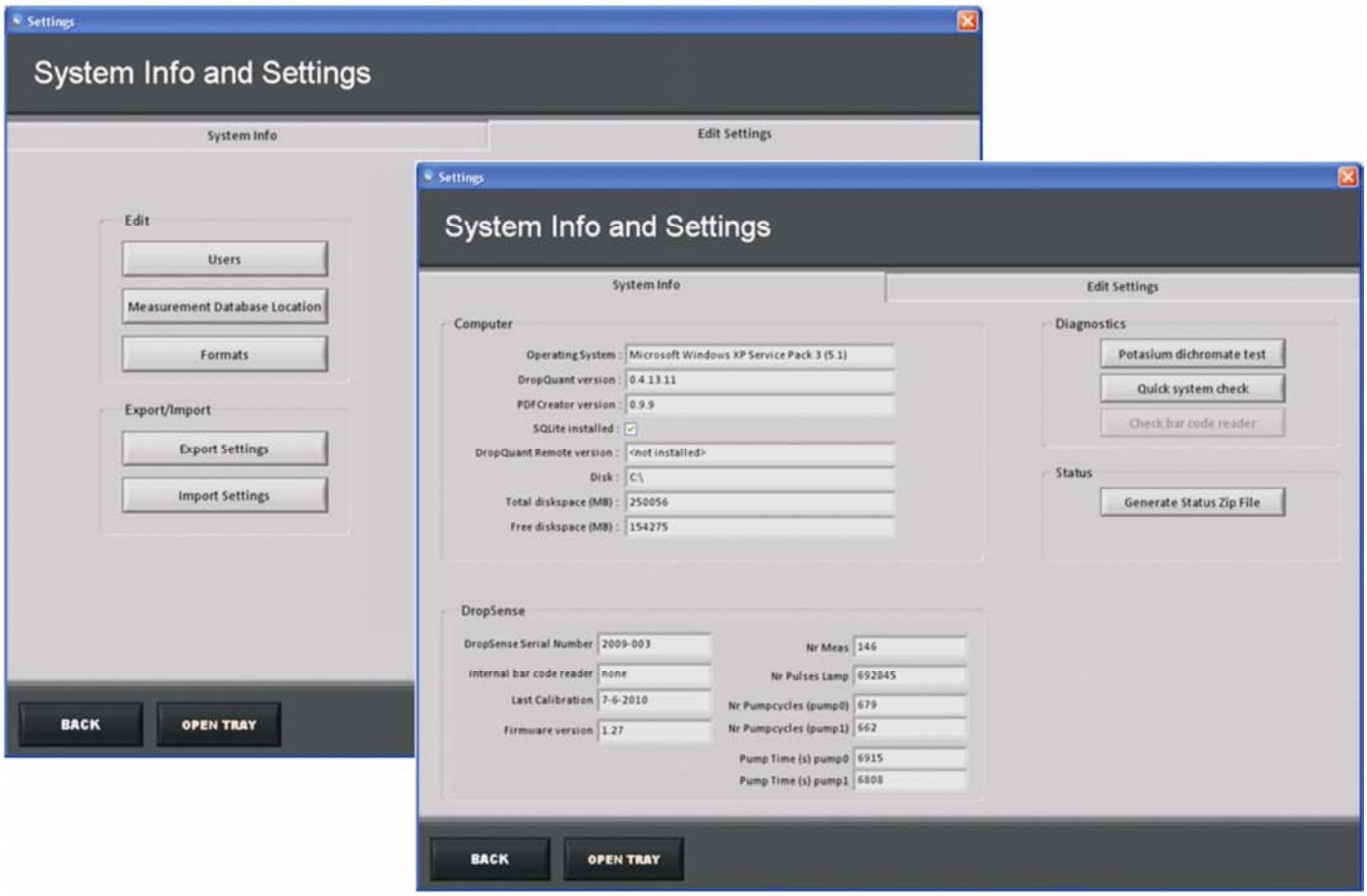
Using the login 'lab manager', additional options appear. The 'system info and settings' button at the left bottom side of the main menu leads to a screen displaying several editing options and an overview of the systems information.

4.1.2.3 System info and settings

The 'Systems info and Settings' button only appears when using the lab manager account or an account with similar entry level. This button opens a new window containing two tabs for systems info, diagnostic tools and software setting options

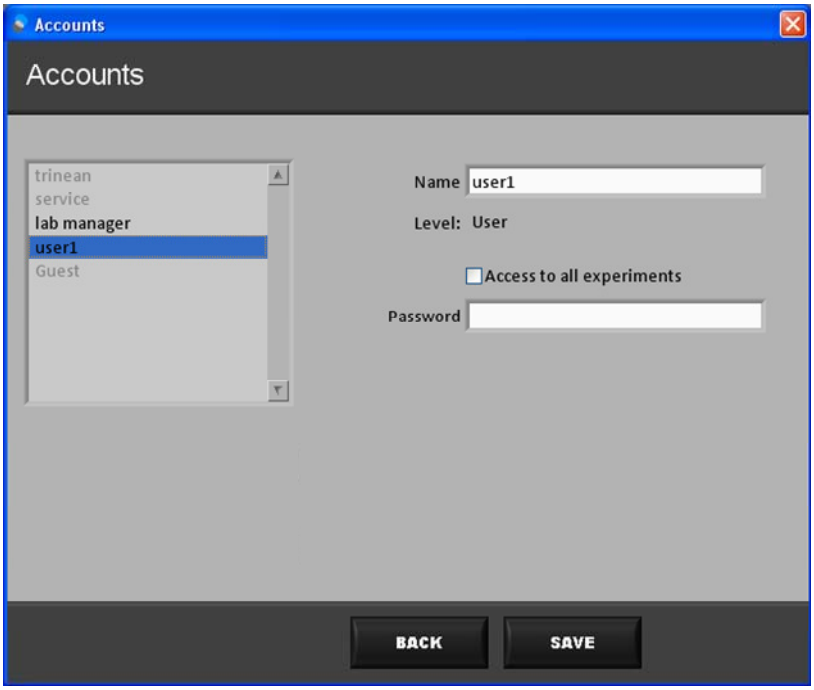
System info

- Detailed description of the PC configuration
- Detailed info on the DropSense96
- Diagnostic checks (further explained in chapter 6)
- A general status of the Dropsense96 can be zipped and sent to the dealer for a first system analysis.



Edit settings

- **Edit Users**: Add or change user accounts and passwords. At the 'lab manager' level, the password can be personalized. During installation, a random lab manager password has been given which can be found on the DropQuant CD included in the package upon arrival. New accounts at the 'user level' can be made and passwords can be given. A new user account has only limited access to the measurement database and can only view his own measurements in the database. However, when user needs to be allowed to see all data in the database, then the 'Access to all experiments' should be turned on. Other accounts (in grey) are not accessible. The 'Guest' account is the default log-in setting and can be used without a password. This account has very limited access to the DropQuant software functions.



- **Edit Measurement Database location:** Alter the location of the raw data measurement database by using the explorer function here.
- **Edit formats:** In the user software, settings can be saved for future use by saving them as a defined format (like import and export formats). In this section, an overview of all used formats will be displayed and a selection of formats can be made by the lab manager so all other user accounts can see and use these formats
- **Export settings:** Export all formats, user database,… These will be saved in a separate folder. This folder can then be used on another PC with installed DropQuant software

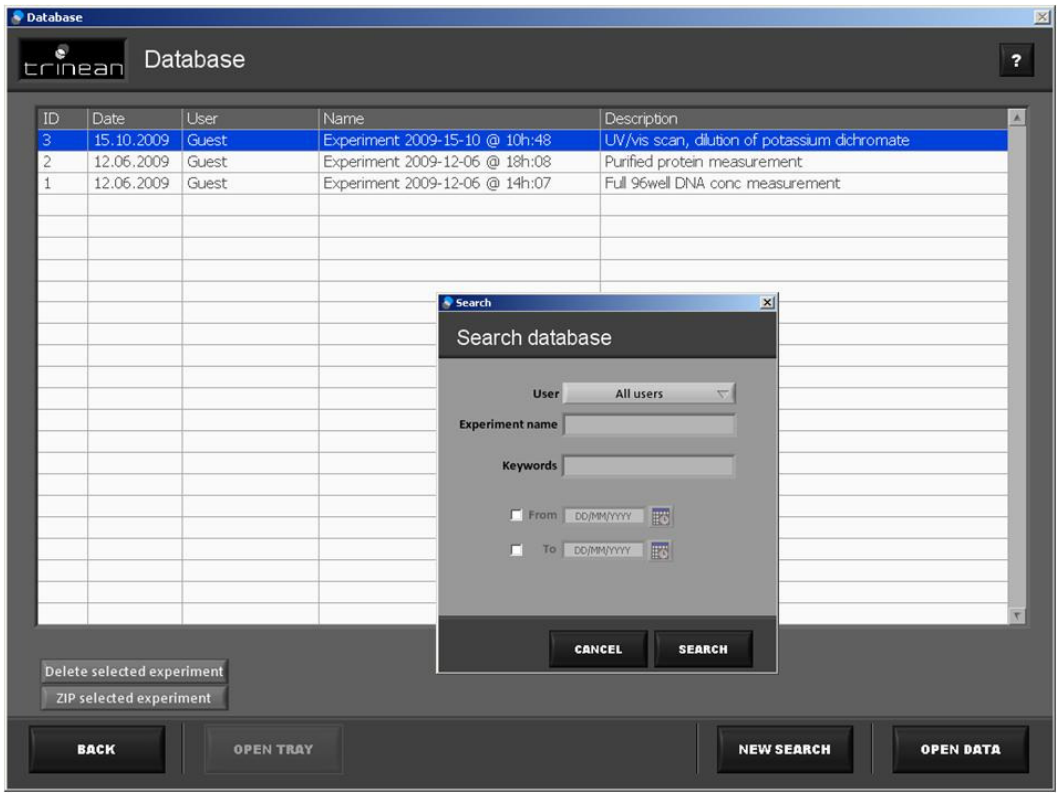
using the import settings button. All saved formats will be activated on the DropQuant software of the second PC.

- **Import settings:** Import folder with saved formats and databases from another PC with DropQuant software.

4.1.2.4 Measurement database

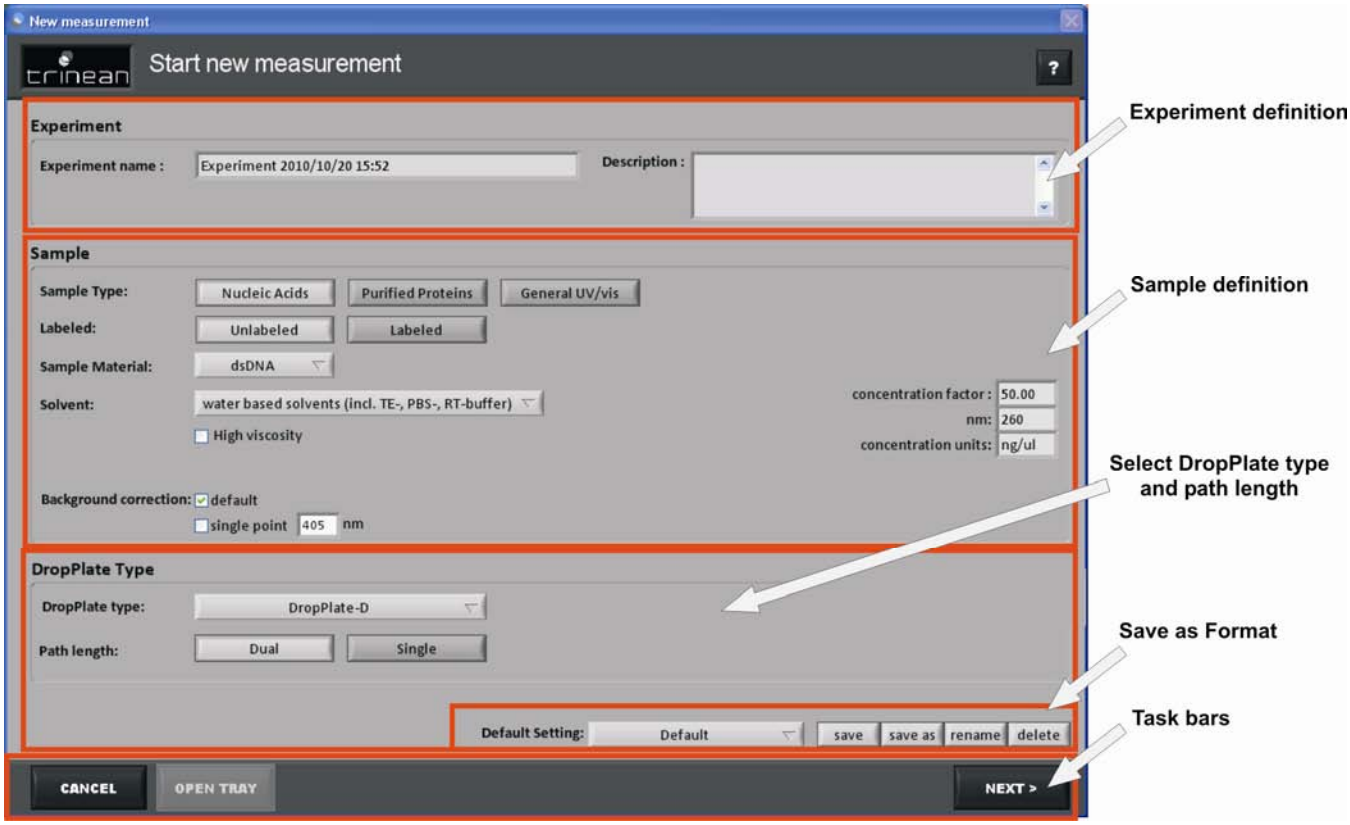
All measurements performed are automatically stored in archive files (location to be determined by the lab manager) and can be opened using the measurement database button on the main menu. All measurements made by a user (log-in) for a given calendar day and time are stored in a single archive file. A unique file extension (.drop) has been given to these files to enable automatic startup with the DropSense96 software. Clicking on the 'new search' button in the task bar will open a search menu allowing searches by user, experiment name, keywords or date.

Select the measurement of interest (blue highlighted) and press the 'open data' button. All information of the measurements will be displayed as a novel measurement (see further) and the spectra are re-plotted for further analysis. Selected measurements can also be deleted or compressed by zipping for other use.



4.1.3 New measurement

This 'new measurement' button on the main menu screen opens the application definition screen to define novel measurements. The operating software has been tailored to meet the life scientist's needs.



Several screen features are built in:

- **Experiment definition:** The user can define the experiment by name and include extra information in the description box. A preset name is generated for every experiment including date and time for easy chronologic storing. All the experiment information will be included in the final experiment report.
- **Sample definition:** Several pre-configurated applications have been included for quick and easy experiment definition. Detailed description of these applications can be found in chapter 5.
    - Nucleic acids (See chapter 5.1)
        - Unlabeled: DNA/RNA concentration (A260) and purity (260/280 and 260/320 ratio) measurements
        - Labeled: DNA/RNA and fluorescent dye labeling density quantification

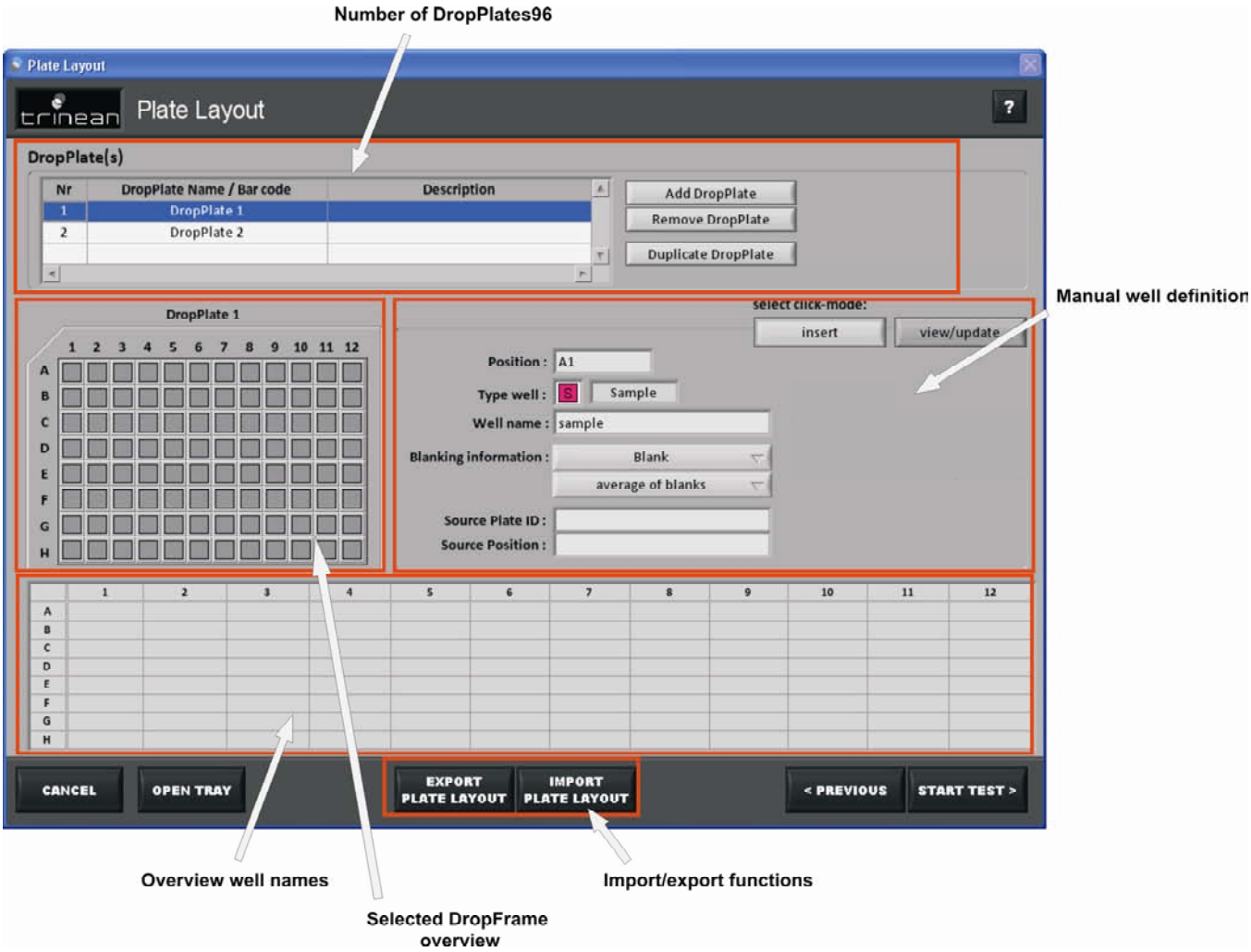
- Purified Proteins (See chapter 5.2)
  - Unlabeled: protein concentration (A280) and purity (260/280 ratio) measurements
  - Labeled: protein and fluorescent dye labeling density quantification
- General UV-VIS (See chapter 5.3)

- **Background selection:** Choice between the default background correction using the spectral range of 400-600 nm or a single wavelength of choice.
- **DropPlate type:** Depending on the application or sample concentration range, a choice of DropPlate-S or DropPlate-D can be made. Both DropPlates are explained in detail in section 3.1. Alternatively, a standard 96well microtiter plate can be read. This selection requires additional information like path length or sample volume and plate brand.
- **Path length selection:** When selecting the DropPlate-D, the user can further choose between a single or dual path length measurement. The single measurement requires 1 µl of sample to perform a single pumping step and a small chamber measurement for high concentration sample analysis (2 to 110 OD for a 10mm path). The dual measurement, requiring 3 µl of sample, uses both measurement chambers for the analysis creating the large concentration measurement range (0.05 to 110 OD for a 10mm path). When using a DropPlate-S, only a single path length measurement is possible. This selection requires a sample volume of 2 µl and generates a measurement range of 0.05-44 OD (for a 10mm path).
- **Save as Format:** When all the above selections have been made, the user can save these in a new format for future use. These formats are account dependent. Only the lab manager account has the possibility to make a format that is available for all users. The formats can be deleted, renamed, …
- **Task bars:** A general task bar is included at the bottom of every screen. This allows the user to cancel the experiment, open the microplate tray at any time and continu to the next software screen.

Within one experiment, all Dropplates need to contain the same sample type as selected on the screen. If the DropPlates contain different sample types, than these DropPlates need to be measured as separate experiments.

4.1.4 DropPlate96 layout

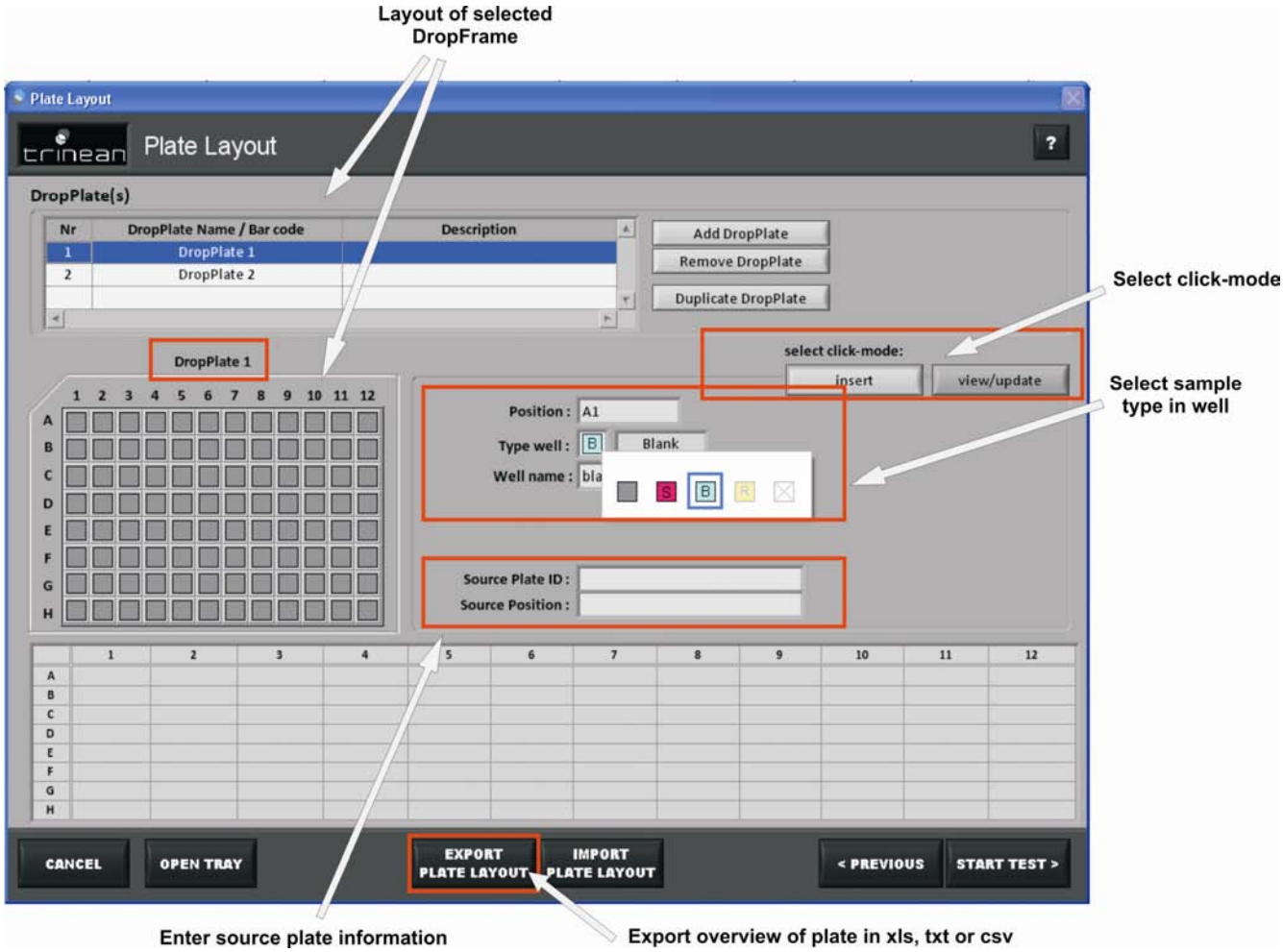
Pressing the 'next' button on the 'Start new measurement' screen will open the screen where information on sample positioning on a 96well display can be entered. The positioning information can be done manually or can be imported from an excel, text or .csv-file.



4.1.4.1 Manual entry of sample ID

Defining the number of DropPlates96:

The number of DropPlate96 (aluminum Dropframe or a full plastic DropPlate96) to be measured can be chosen with the 'add DropFrame' button and specific names can be given. Unnecessary DropPlates96 can also be removed. Furthermore, a DropPlate with all the positioning information can be duplicated, creating a new DropPlate with identical information. When selecting a DropFrame96 in the table (colored blue), the 96well display underneath will show an overview of this particular DropPlate96.



Select click-mode

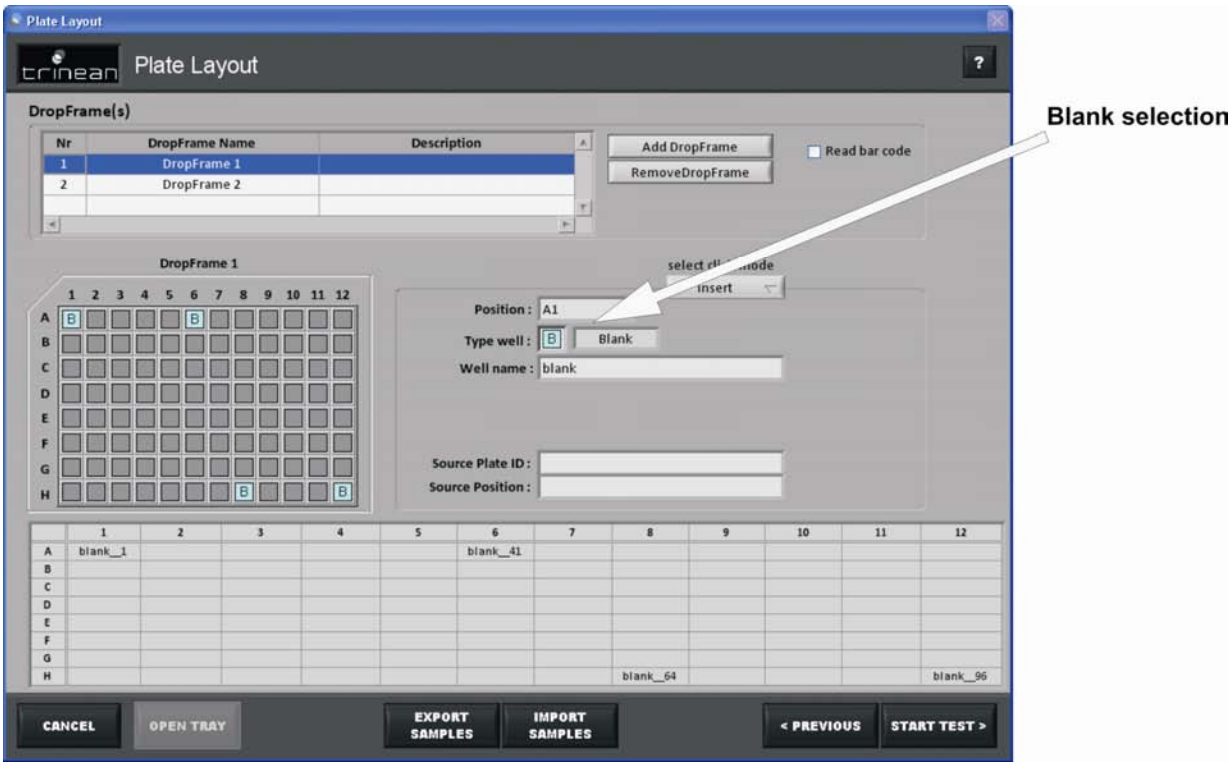
Select click-mode contains two choices: 'insert' or 'view/update'. The insert mode will directly insert the information below (type well/well name,…) in the selected well in the 96 matrix on the left. So, the user can change the information for a well first and by clicking on the well, the information is stored. With the view/update mode, the user can click on a well and obtain the information of this well without changing it. If the information of the well needs to be chanced, the user can change the info (type well/well name,…) and then click 'update' above right on the screen.

Type well

Three well types can be chosen by clicking on the colored square : **Empty** (grey), **Blank** (blue), **Sample** (red). The yellow reference cannot be chosen yet but will be used in future applications. The empty option can be used to erase errors. However, a safety procedure is implemented in the software to avoid erasing blank wells.

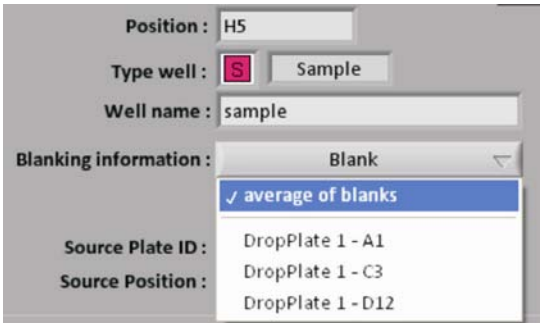
Blanking information

It is recommended to position the blank references first, then the samples.



A choice can be made between 'Autoblank' (the background absorbance is automatically set to zero) or 'Blank' (a blanking reference included in this experiment).

When several blanking solutions are used and positioned on one or multiple DropPlates96 for the experiment, then these different blanks can be chosen as reference for any samples on any plate of the experiment. The software automatically presents the entered blank wells where the user can select from.

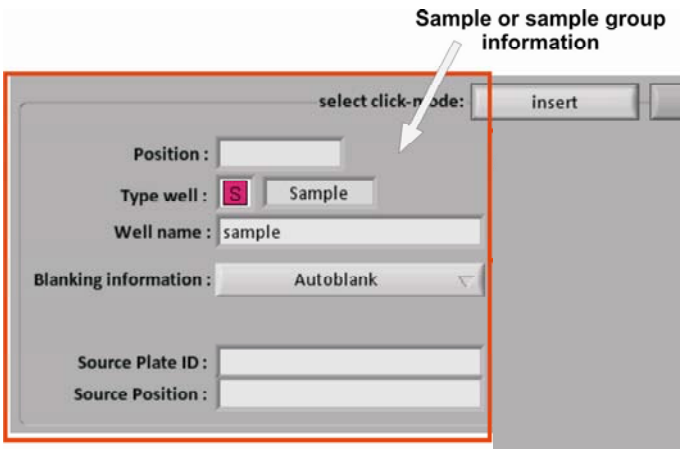


Another option in the software is to take the average data of all blanks included in the experiment and use that info as blanking data for the samples.

For sensitive measuring of low concentrated nucleic acid samples (example: dsDNA < 100ng/µl). We recommend to always use a blank sample. The 'autoblank' method is a software adjustment of the spectra (background correction) and can be less accurate then when using a proper blank sample.

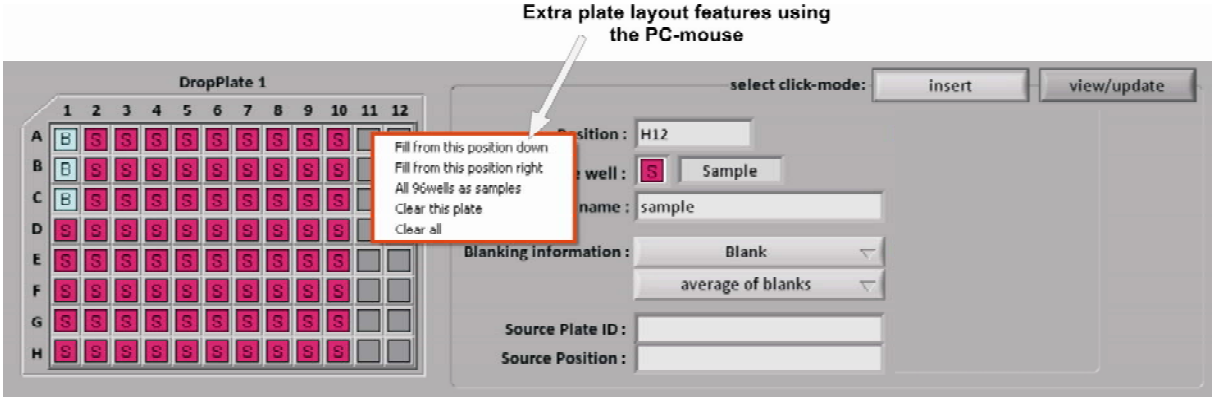
Sample information

Select sample as 'Type well'. Change the sample name if needed and select what type of blanking is used for this sample or group of samples. A source plate location per sample can also be entered here. A convenient way of fast well identification of a sample group is by selecting an area on the DropPlate96 by dragging with the computer mouse. Alternatively, one by one can be selected by clicking on the correct position on the 96well plate. All selected wells will contain the information shown on the left side (sample/blank/empty, choice of blank,…). The name of these defined wells can be added to the sample in the box next to 'Well name' or by entering the name in the matching well space in the table at the bottom of the screen by double clicking on the well space. As default, samples will be given numbered names starting with 'sample_1' in well A1 to 'sample_96' in well H12. An overview of all well names is given in the table at the bottom of the screen.



Additional options

Using the right PC-mouse button on the 96well layout figure will open additional plate layout features to fill in full rows, colums or a full 96well plate and delete the plate layout.



Once the full plate layout has been completed, this information can be saved for future use. The 'export plate layout' button at the bottom opens a pop-up window offering the choice of several formats (txt, xls, csv) to save the information. When selecting Excel, an 96well matrix overview is made in the sheet. This can be useful in data analysis or reports in the experimental log book.

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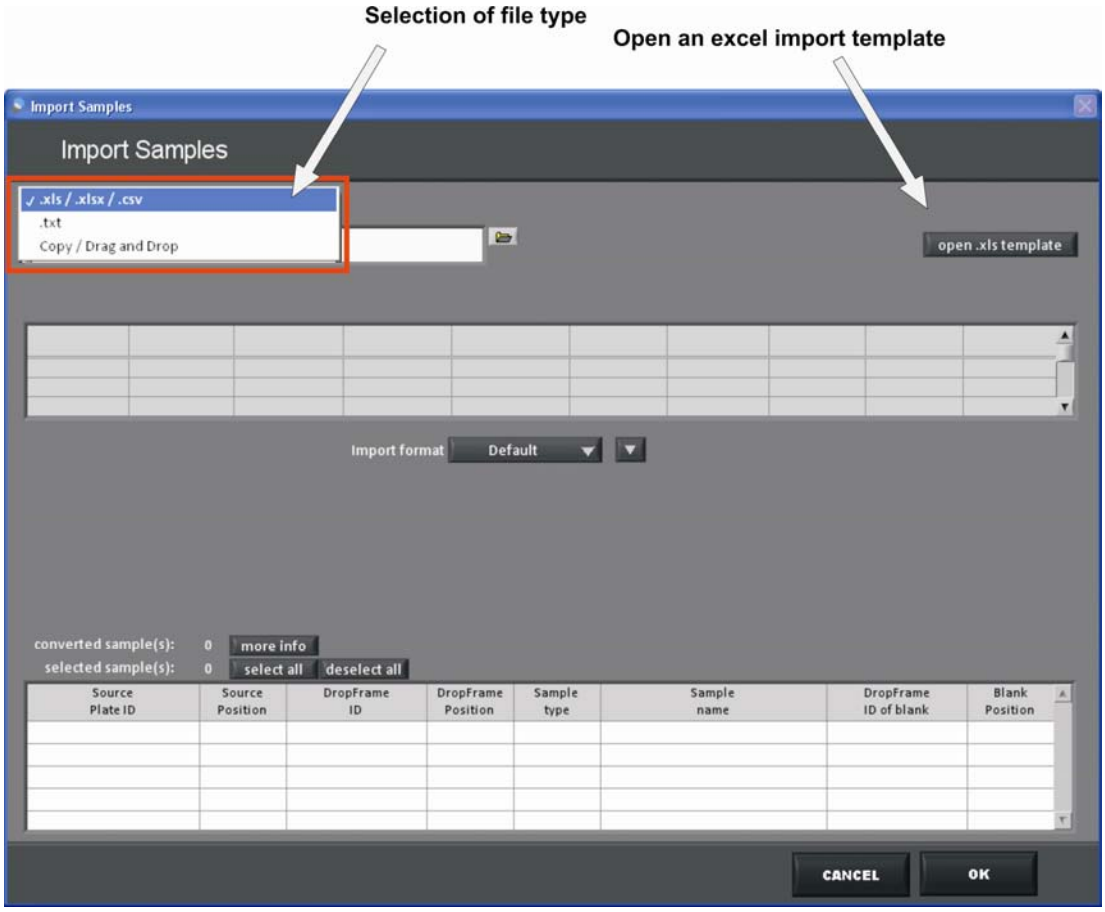
Plate Layout

DropFrame 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank__1	blank__9	blank__17	blank__25	blank__33	blank__41	blank__49	blank__57				
B	sample__2	sample__10	sample__18	sample__26	sample__34	sample__42	sample__50	sample__58				
C	sample__3	sample__11	sample__19	sample__27	sample__35	sample__43	sample__51	sample__59				
D	sample__4	sample__12	sample__20	sample__28	sample__36	sample__44	sample__52	sample__60				
E	sample__5	sample__13	sample__21	sample__29	sample__37	sample__45	sample__53	sample__61				
F	sample__6	sample__14	sample__22	sample__30	sample__38	sample__46	sample__54	sample__62				
G	sample__7	sample__15	sample__23	sample__31	sample__39	sample__47	sample__55	sample__63				
H	sample__8	sample__16										

4.1.4.2 Import of plate layout by file (CSV, XLS and TXT import)

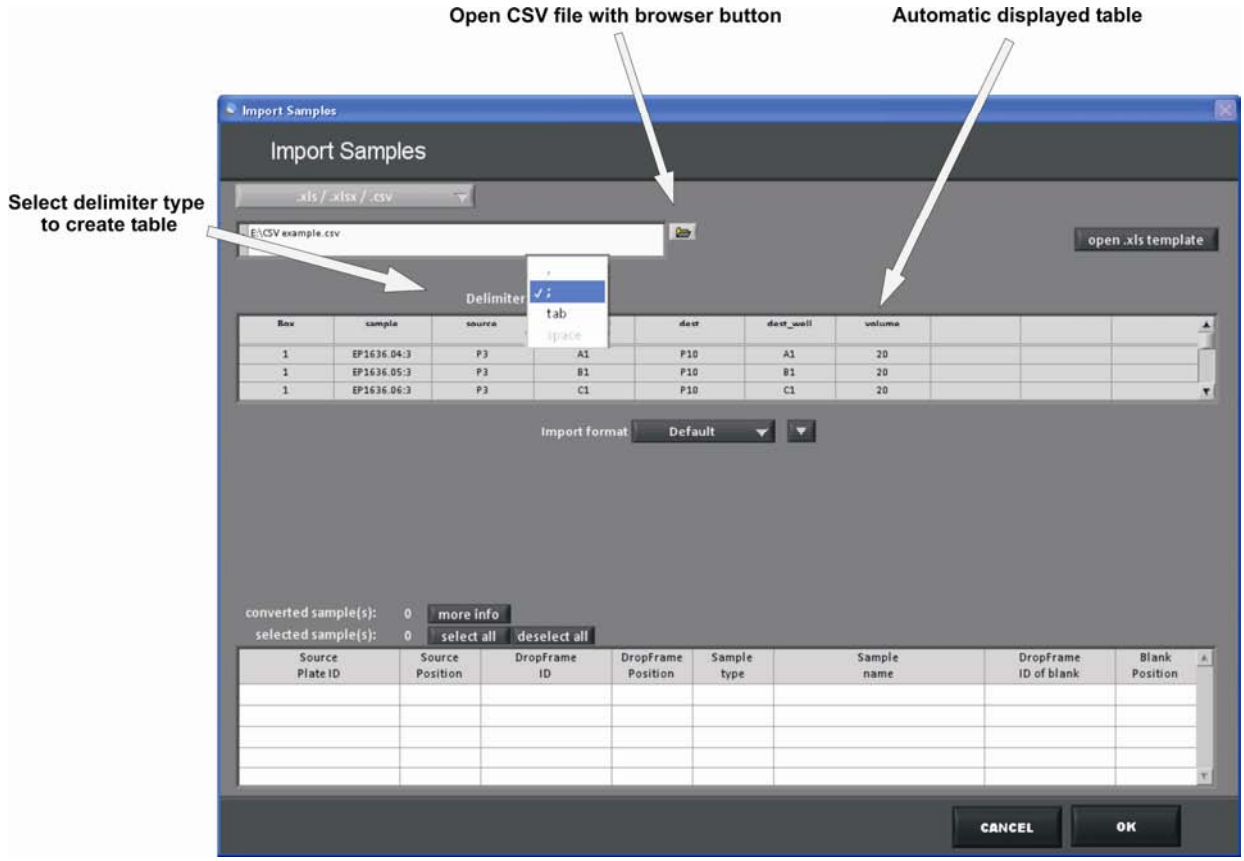
An import function ('Import Plate layout') is foreseen at the bottom of the 'Plate Layout' screen for fast and easy entering of plate layout information. Several types of files (excel, txt and csv) can be imported and the data can be reformatted into a dedicated DropQuant software table for quick plate layout configuration.

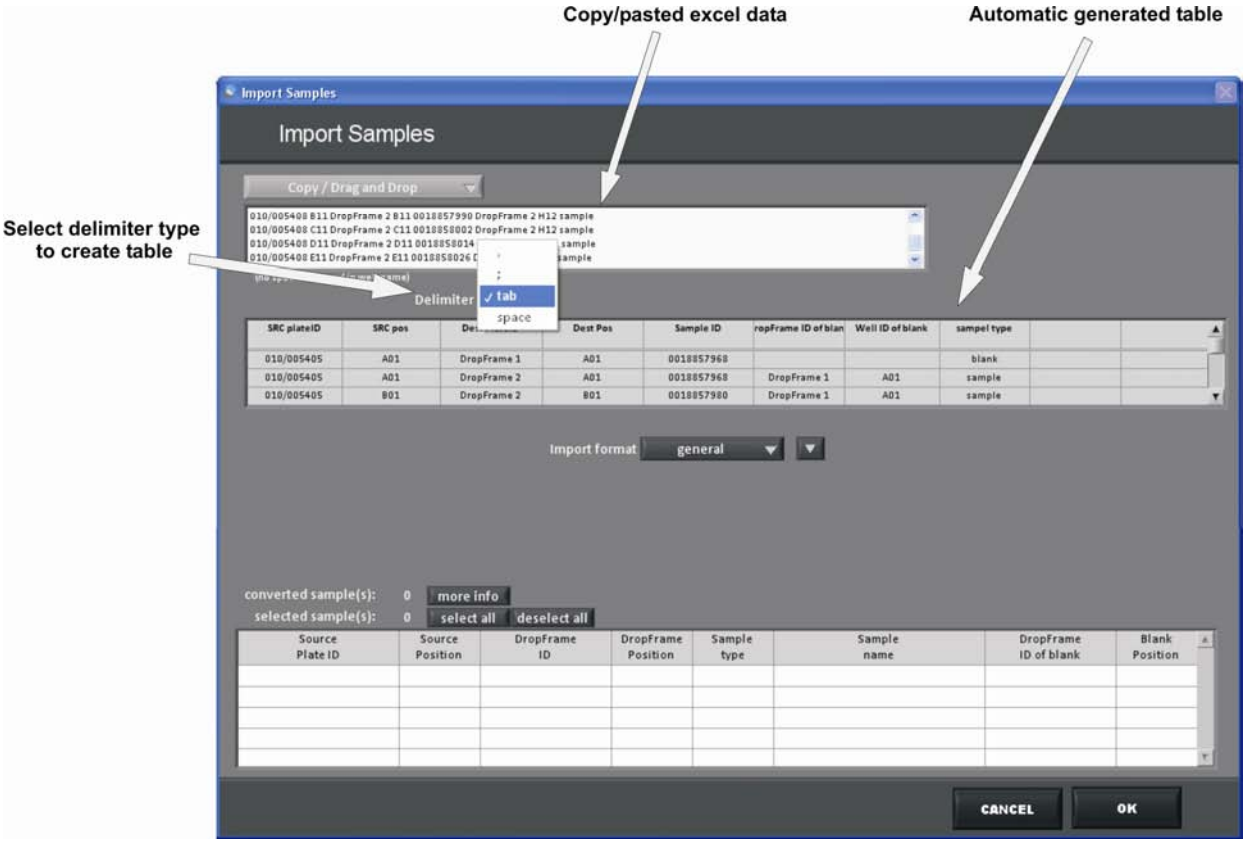


Excel, txt or comma-separated values (CSV) file selection

CSV files can be selected using the browse button and the table underneath will be filled in automatically. The appropriate delimiter needs to be selected to ensure that the table is filled in properly.

Txt files can also be uploaded by direct selection using the browse button or by the 'copy/drag and dropped' option. Like CSV files, an appropriate delimiter needs to be selected. Excel files can also be directly added in the import screen via the on-screen browse button. After importing the information from the excel file, the associated table below is filled in automatically. Selection of the appropriate sheet of the excel file is needed for proper table formation.


Open CSV file with browser button
Automatic displayed table
Select delimiter type to create table
Import Samples
Import Samples
.xls / .xlsx / .csv
E:\CSV example.csv
open .xls template
Delimiter
;
tab
space
Box
sample
source
dest
dest_well
volume
1 EP1636.04:3 P3 A1 P10 A1 20
1 EP1636.05:3 P3 B1 P10 B1 20
1 EP1636.06:3 P3 C1 P10 C1 20
Import format
Default
converted sample(s): 0
more info
selected sample(s): 0
select all
deselect all
Source Plate ID
Source Position
DropFrame ID
DropFrame Position
Sample type
Sample name
DropFrame ID of blank
Blank Position
CANCEL
OK

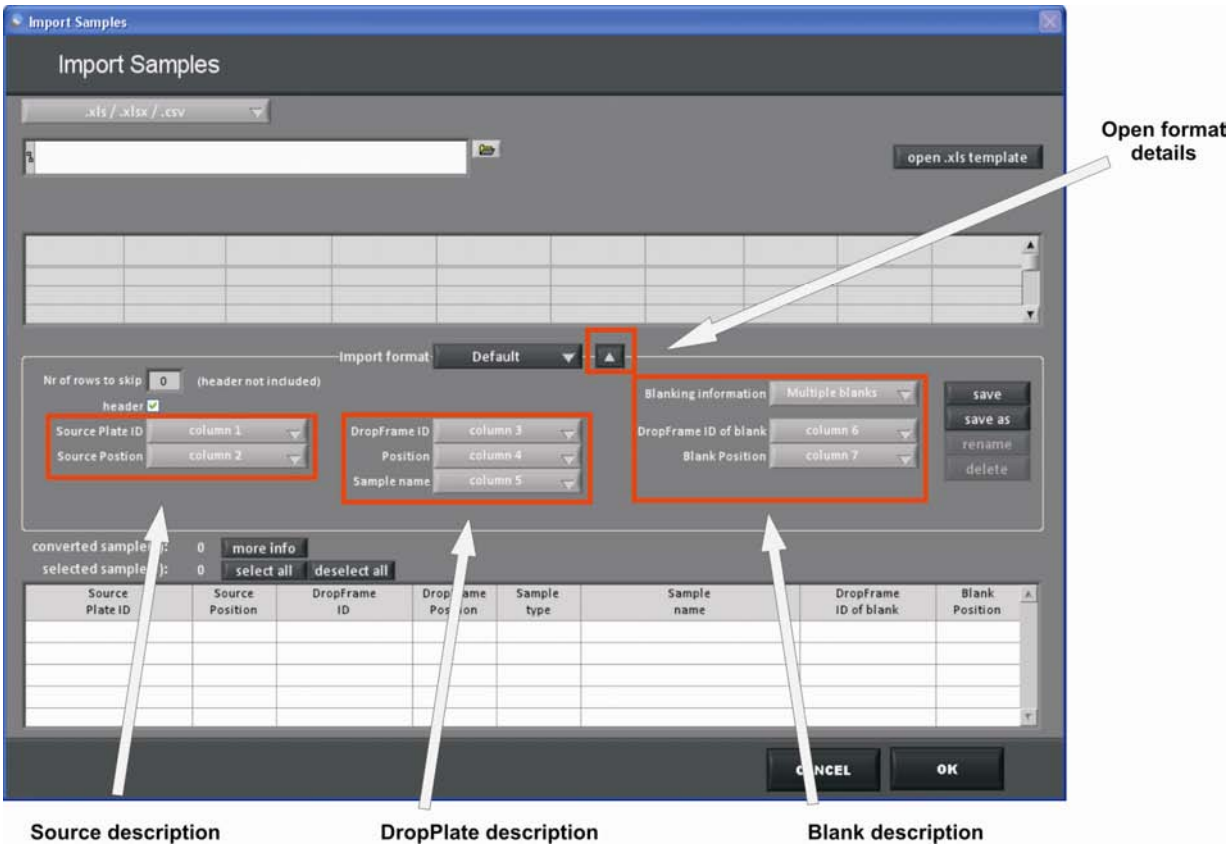

Copy/pasted excel data
Automatic generated table
Select delimiter type to create table
Import Samples
Import Samples
Copy / Drag and Drop
010/005408 B11 DropFrame 2 B11 0018857990 DropFrame 2 H12 sample
010/005408 C11 DropFrame 2 C11 0018858002 DropFrame 2 H12 sample
010/005408 D11 DropFrame 2 D11 0018858014
010/005408 E11 DropFrame 2 E11 0018858026
Delimiter
;
tab
space
SRC plateID
SRC pos
Dest Pos
Sample ID
Well ID of blank
sampel type
010/005405 A01 DropFrame 1 A01 0018857968 blank
010/005405 A01 DropFrame 2 A01 0018857968 DropFrame 1 A01 sample
010/005405 B01 DropFrame 2 B01 0018857980 DropFrame 1 A01 sample
Import format
general
converted sample(s): 0
more info
selected sample(s): 0
select all
deselect all
Source Plate ID
Source Position
DropFrame ID
DropFrame Position
Sample type
Sample name
DropFrame ID of blank
Blank Position
CANCEL
OK

Alternatively, the information in the excel table can be 'copy/drag and dropped' directly from the opened excel sheet into the blank window on the import sheet. This information will also be used to complete the associated table below.

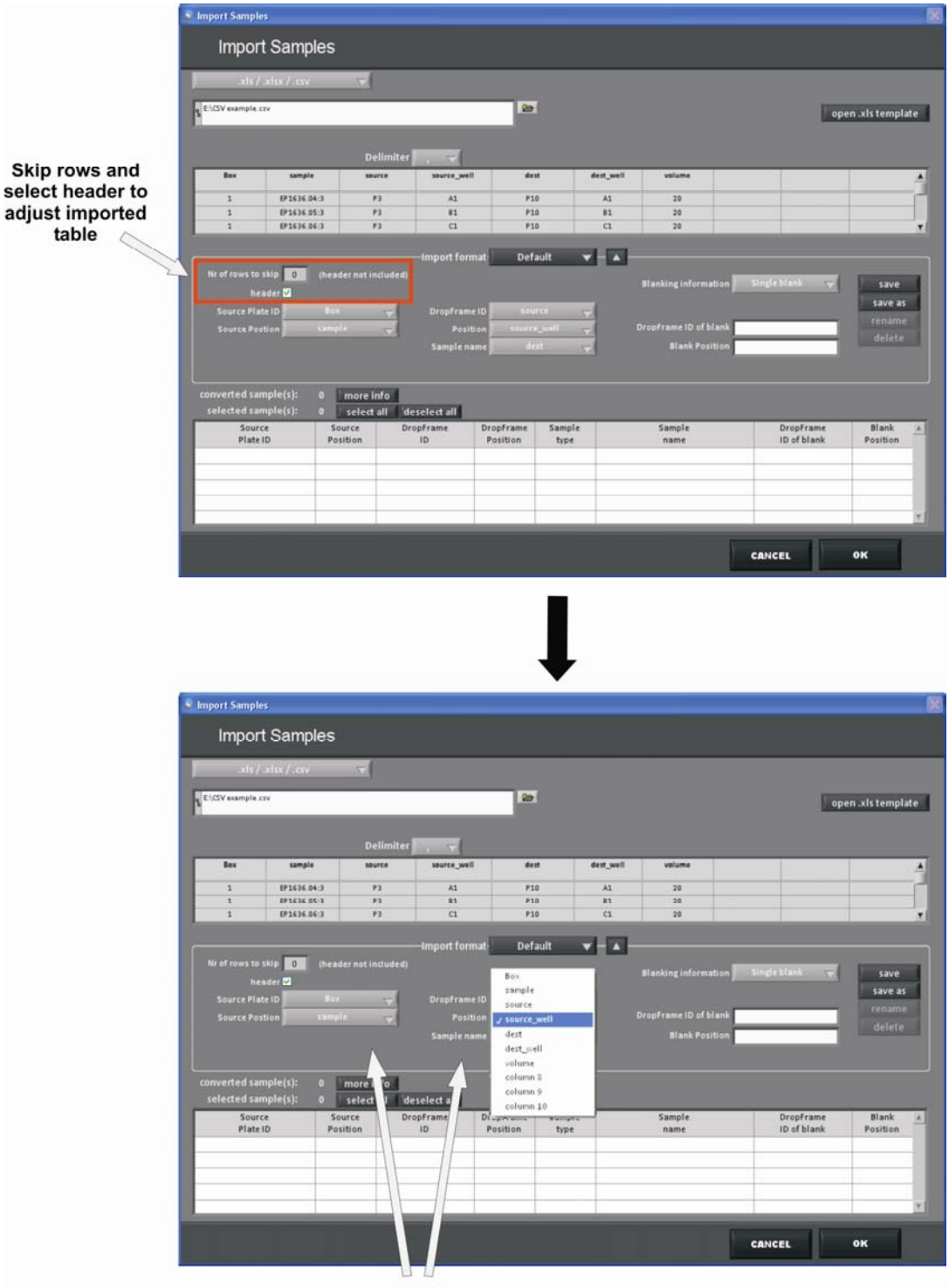
When no excel table is available, press the 'open .xls template' button to open an example sheet with defined columns. These columns can be deleted or others can be added to create a customized import excel sheet.

Import format design

After entering excel, txt or CSV data, a import format has to be designed. The default 'general' format has been designed in combination with the excel template. This excel template can be viewed by clicking in the 'open .xls template'. The small button next to 'general' opens the detailed formatting options. Now, several drop down buttons are shown, devided in three sub groups: source description, DropPlate description and Blank information. Next to the latter sub group, several buttons are foreseen to add new formats, save, delete or rename them. Using the 'add' button, a new format can be defined compliant with the user specific imported data file.



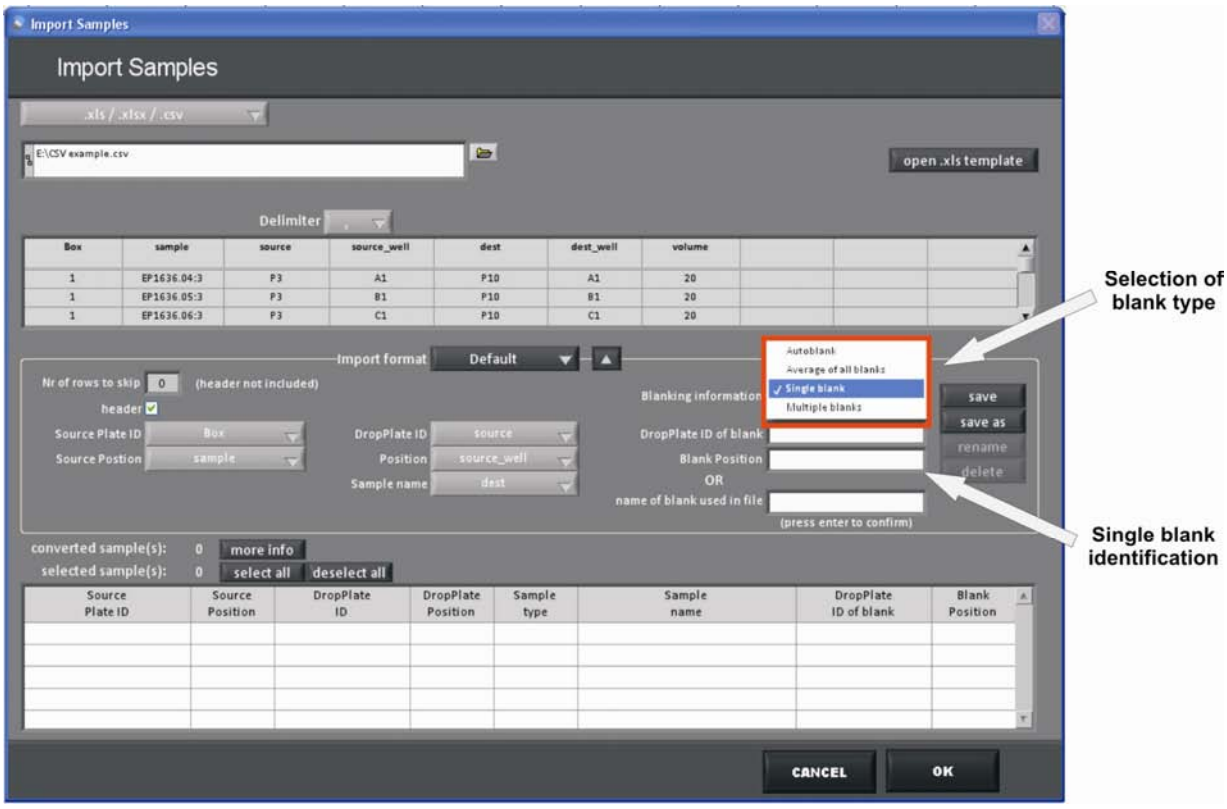
After giving the new format a name, the columns as shown in the preformed table above must be assigned to the appropriate definition (Source plate ID, Source position, sample name,…). If the automatic imported table contains rows before the required data and/or a table header, then this information can be specified for an optimal table view. The appropriate rows need to be selected containing source information and DropPlate information.



Assign columns to the appropriate description

A separate blank selection is provided including different blanking options: ‘autoblank’, ‘average of all blanks’, ‘single blank’ and ‘multiple blanks’. Choose the option ‘multiple blanks’ when different blanking solutions are used for separate sample groups in the experiment. In that case, two extra columns need to be present in the imported file: one column shows the DropPlate ID and the other the well position of the blank on that DropPlate. An example can be seen by opening the ‘open xls template’ button. When only one blank is used for the complete experiment, then choose the ‘single blank’ option and type in the blank well position and the specific DropPlate ID on which the blank is loaded or type in the name of the blank (software will locate the correct position from the name). When a single blanking solution is loaded more than once and the average of those measurements should be used as blanking information, then select the ‘average of all blanks’ and type in the blank name. The same name must be used for all the loaded blank wells, so that the software can locate them all and determine the average of all these blanks.

Note: The ID of the DropPlate containing a single blank can be entered or an external bar code reader can be used to read the bar code on the side of the DropPlate.



5. Applications

5.1 Nucleic acid quantification and purity determination

5.1.1 Theory: DNA/RNA concentration and purity determination

5.1.1.1 DNA/RNA concentration measurement

Nucleic acid samples can simply be checked for concentration and quality using the DropSense96 spectrophotometer. Nucleic acids have an absorption maximum at 260 nm (A260) and the spectrographic reading at this wavelength is the most common method for detecting dsDNA, ssDNA, RNA and oligonucleotides in a solution. The nucleic acid concentration is calculated using the Beer-Lambert law (see below), which predicts a linear change in absorbance with concentration. Using this equation with a 1 cm path length, an A260 reading of 1.0 is equivalent to ~50 ng/µl dsDNA, ~33 ng/µl or ~40 ng/µl single-stranded RNA. .

Beer-Lambert Law:

$$A=\varepsilon.c.l$$

Where:

A = absorbance at a particular wavelength

ε = the extinction coefficient

c = concentration of nucleic acid

l = path length of the spectrophotometer cuvette

The DropSense96 performs a optical OD measurement, using path lengths insured by the choice of DropPlate to enable quantification of nucleic acid samples with a wide concentration range without need for dilution.

The absorbance data are archived in the database and displayed on the software screen after measurement. In the nucleic acid application, the software chooses automatically which path length generates the best absorbance values and normalizes this measurement to a 1.0 cm (10.0 mm) path. These are displayed in the software for further data analysis.

5.1.1.2 DNA/RNA purity determination

Residual cellular contaminants like proteins or compounds used in the DNA/RNA preparation frequently remain present in the DNA solution and often interfere with the measurement at 260 nm, leading to incorrect results. Both protein and DNA absorb UV light, but have different absorbance curves. The peak of light absorption for DNA is at 260 nm, while proteins absorb strongly at 280 nm, mainly due to tryptophan and tyrosine side chains. Therefore, the purity of

a DNA sample can be calculated by examining the ratio of the two absorbance values. A260/A280 values of ~1.7 to 1.8 predict “clean” DNA; good quality RNA will have a 260/280 ratio of ~1.8-2.0. Lower values may be indicative of significant protein, phenol or other aromatic compound contamination.

However, the A260/280 ratio is not always an accurate representation of DNA purity. Other contaminating substances like EDTA and carbohydrates have a low 280 nm absorption but absorb UV light around 230nm. Furthermore, some proteins containing few aromatic residues have little absorbance at 280 nm, while all proteins have a clear absorption peak at 228 nm due to their peptide bonds. This makes the 260/230 ratio often a more constant indicator of the presence of protein in a nucleic acid sample. Therefore, absorbance readings measured both at 230 nm and at 280 nm provide a more accurate estimate of contaminants that may be present in nucleic acid samples. The ratio of the A260/A230 should be ~1.8 or greater since nucleic acids have an absorbance minima at 230 nm.

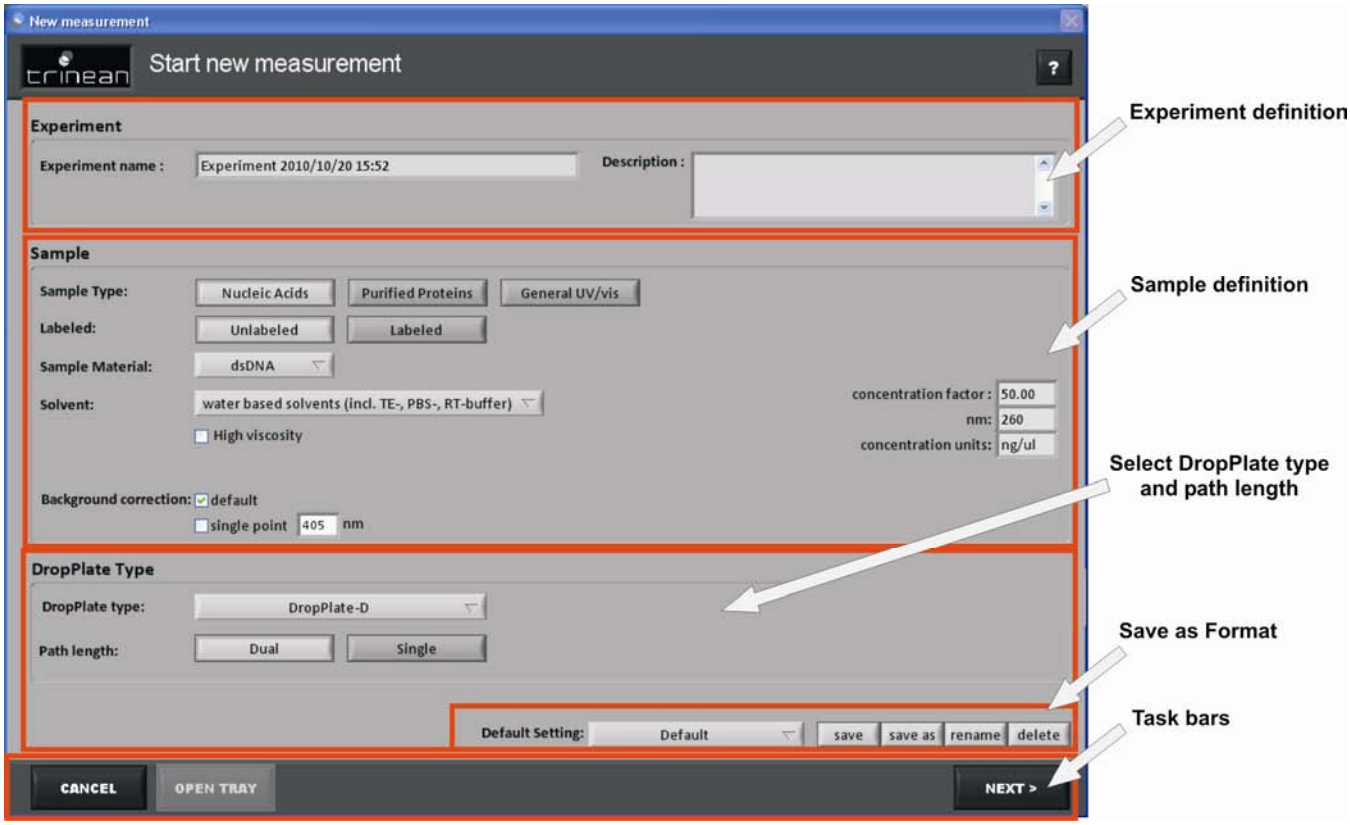
It is recommended to use the absorbance readings at A260, 280, and 230 and examining both A260/280 and A260/230 ratios for every sample. As a general rule, a 260/280 ratio of ~1.8 and a 260/230 ratio of ~2.0 or greater predict 'clean' DNA.; good quality RNA will have a 260/280 ratio of ~1.8-2.0 and an OD ratio 260/230 of ~2.0 or greater.

The 260/280 ratio can differ depending on the spectrophotometer used. It is dependent on both the characteristics of the sample (pH, ionic strength) and the wavelength accuracy of the spectrophotometer used. Since the DNA absorption peak shows a steep slope at 280 nm, a slight shift in wavelength accuracy (+/- 1 nm) can result in ~0.2 change in the 260/280 ratio.

The solution properties pH and ionic strength can also affect the 260/230 and 260/280 ratio's. Therefore, we recommend using a buffered solution like TE (pH 8.0) as both the nucleic acid diluent and the blanking solution during the measurement. Pure water often has an acidic pH, lowering the 260/280 ratio, while TE buffer has an intrinsic UV absorption below 240 nm.

5.1.2 Input of nucleic acid sample data

Continue on the 'Start new measurement' screen.



Experiment definition.

The default experiment name includes date and time for easy chronologic overview of the database. The experiment name can be changed and a experiment description can be added. Both are displayed in the database section.

Sample Type:

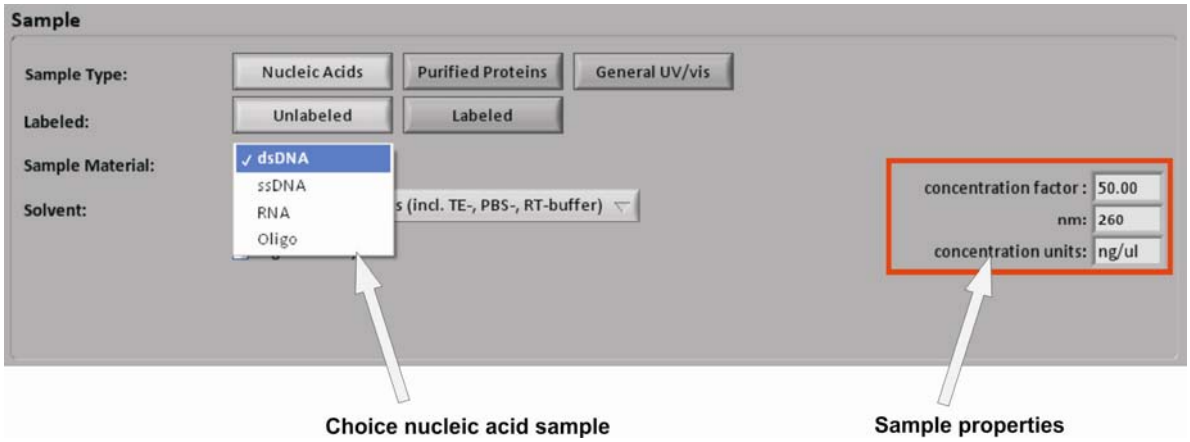
Select 'Nucleic Acids' on the 'Start new measurement' Menu (the button turns light gray upon selecting).

Labeled:

Make a choice between 'Unlabeled' for general nucleic acid quantification or 'Labeled' when a fluorescent label is present and the labeling efficiency needs to be determined.

Sample material:

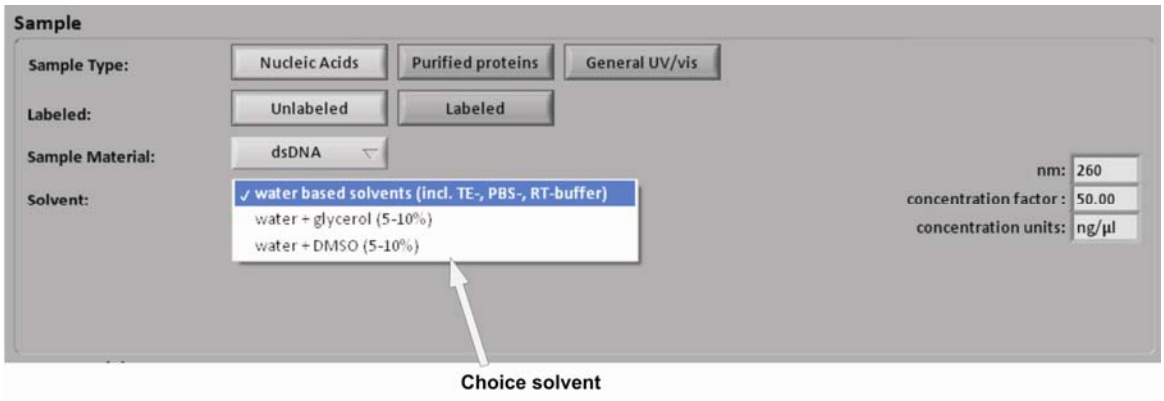
Select the type of nucleic acid in this experiment. The user can select 'dsDNA', 'ssDNA', 'RNA' and 'Oligo'. For every selection, the sample properties used for the concentration calculation are shown on the right side.

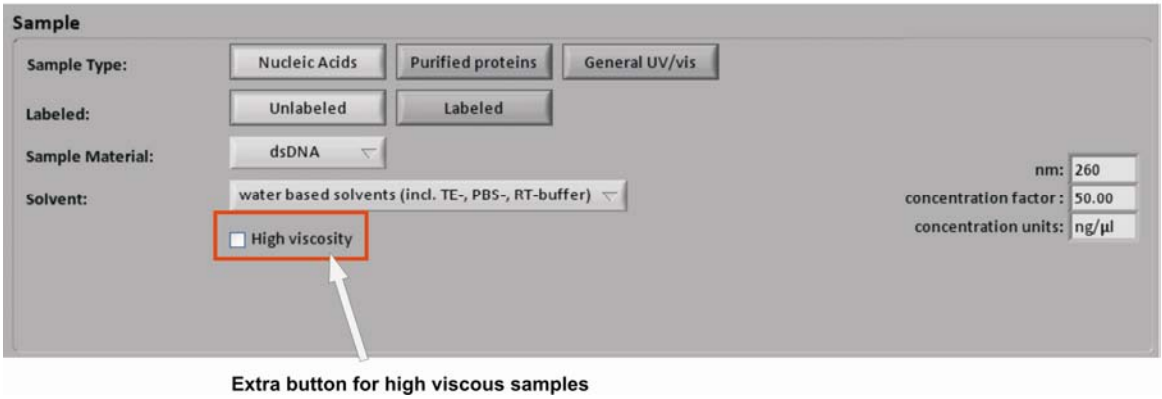


Unique features for measurement of oligo nucleotides are included in the software. Different oligo's can be measured in the same experiment. For each oligo the molecular weight, extinction coefficient and concentration factor is measured (see 5.1.4).

Solvent:

Select the solvent used for the samples to be measured. This solvent information is used in the DropQuant software of the DropSense96 to fine-tune the pressures used by the vacuum pumps inside the instrument guaranteeing precise and controlled filling of the microfluidic structures. All water-based solvents containing low amounts of salts and buffers are grouped in one option since these substances don't affect the pumping properties.

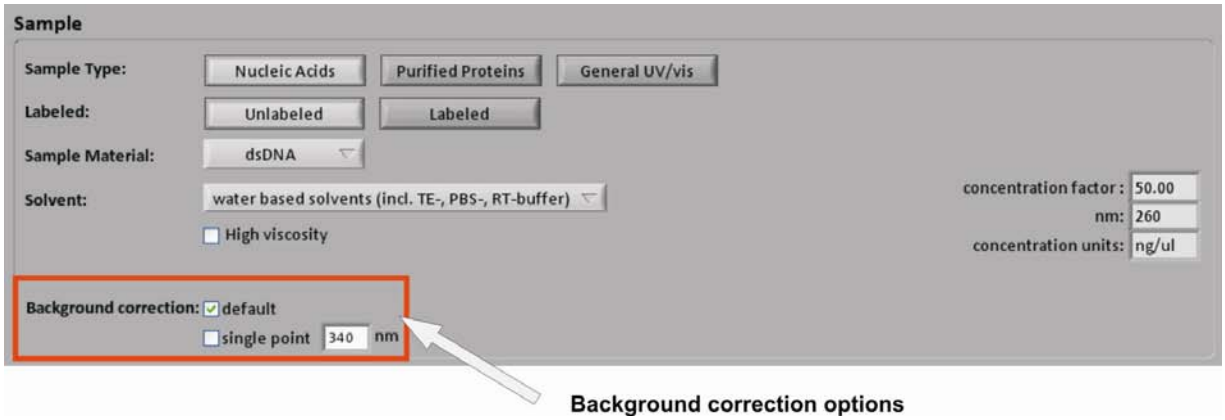




Field tests have shown that genomic DNA, lambda DNA and highly concentrated nucleic acid samples can be very viscous and non-homogeneous. This can hamper both precise pipetting of the DNA samples as well as precise transport of the sample in the microfluidic DropPlate. An extra button for high viscous samples can be used, so the pumps will work at higher pressure and longer duration to fill both measuring cuvettes on the DropPlates correctly. This implies that the total reading time of an experiment is longer than a similar experiment with normal samples.

When the sample is still too viscous, the dual measurement can fail. This will be indicated by an orange or red color indication when the results are shown (see further). Heat the sample to 55°C and then gently vortex to minimize the viscosity and try to measure again.

Background correction:

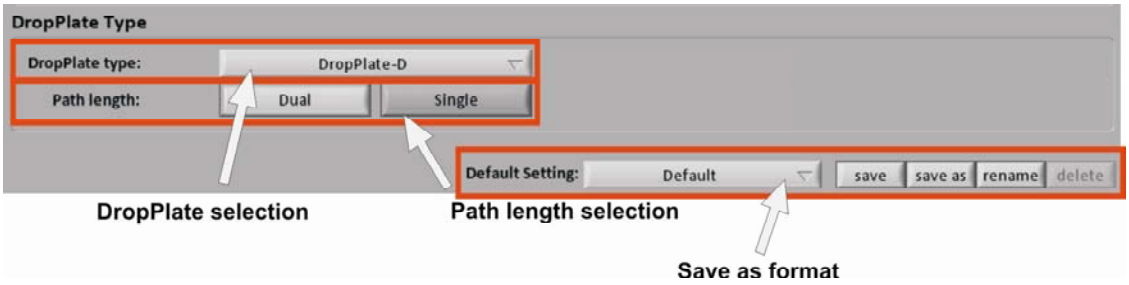


Background correction options

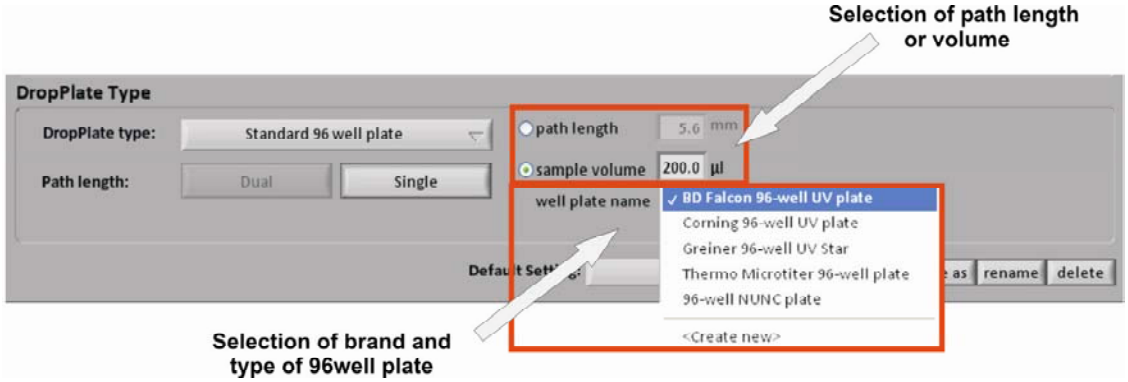
A choice can be made between two types of background corrections. The default background correction determines a linear fit through the 400-600nm part of the spectrum and subtracts this background curve from the full OD range.

The second option is to choose a single wavelength (most common used wavelengths by 'classic' spectrophotometers are 320, 340 and 405 nm). The absorbance value of that wavelength will be subtracted from all wavelength data, so the full spectrum will be set so that the background wavelength of choice will be zero. During the data analysis, the background correction can be altered again or turned off for detailed analysis.

DropPlate selection



Depending on the application or sample concentration range, a choice of DropPlate-D or DropPlate-S can be made. Both DropPlates are explained in detail in section 3.1. Alternatively, a standard 96well microtiter plate can be used. This selection requires additional information like path length or sample volume combined with plate brand and type in order to calculate the correct path length. Additional plate brands and types can be entered by selecting <Create new> from the drop down list.



Path length selection

When using **DropPlate-D** disposables, the user can select between a single or dual path length measurement (see 3.1.2). The single measurement requires 1 µl of sample to perform a single pumping step and a small chamber measurement for high concentration sample analysis (100-5500 ng/µl dsDNA). The dual measurement, requiring 3 µl of sample, uses both measurement chambers for the analysis creating the large concentration measurement range (5-5500 ng/µl dsDNA).

When using a **DropPlate-S**, only a single measurement is possible. This consumable requires 2 µl of sample and performs a single measurement with a measurement range of 10-1200 ng/µl dsDNA.

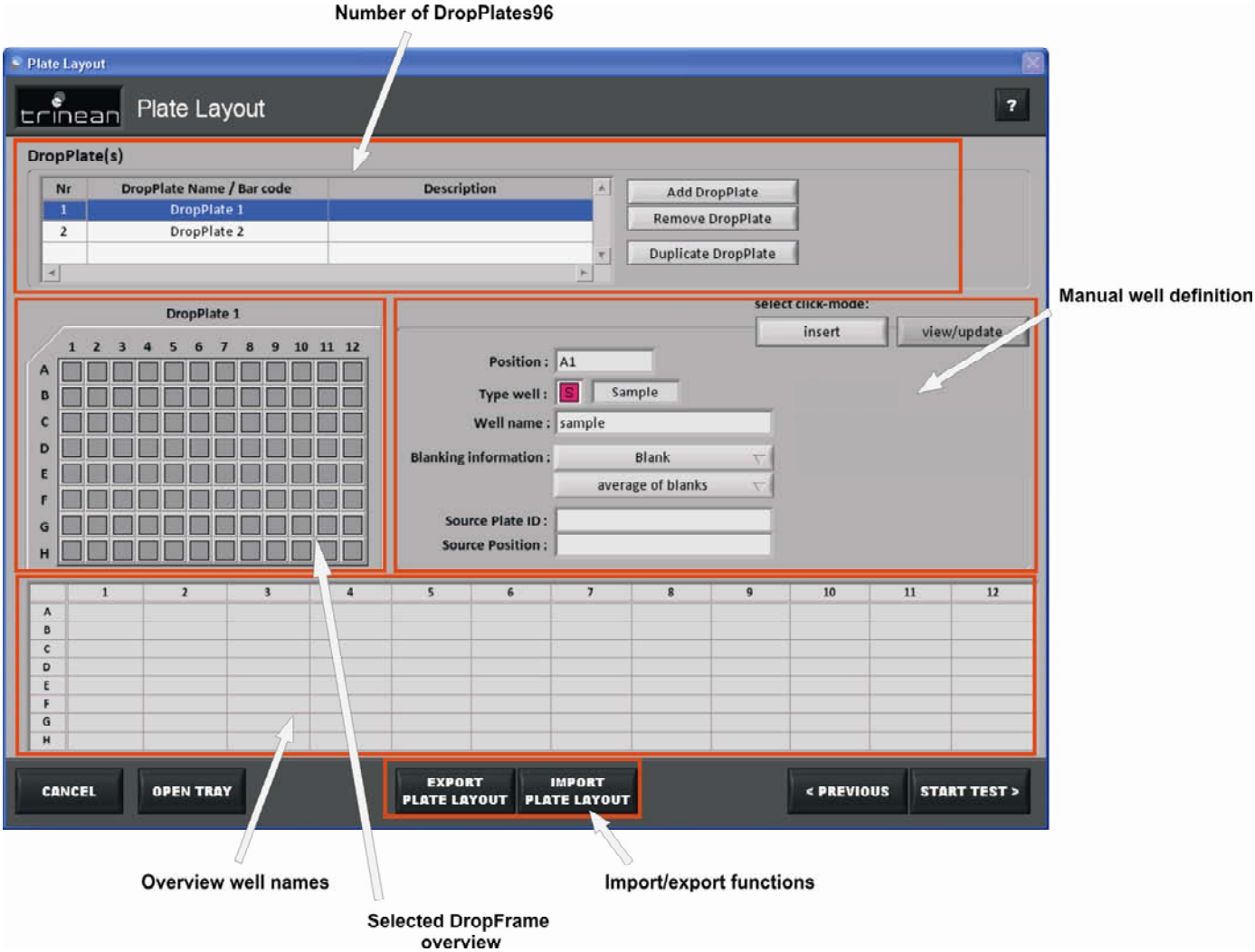
Save as a format:

When all the above selections have been made, the user can save these in a new format for future use. These formats are account dependent. Only the lab manager account has the possibility to make a format that is available for all users. The formats can be deleted, renamed, …

When all sample properties are entered, press the 'next' button on the task bar below

5.1.3 DropPlate96 sample layout

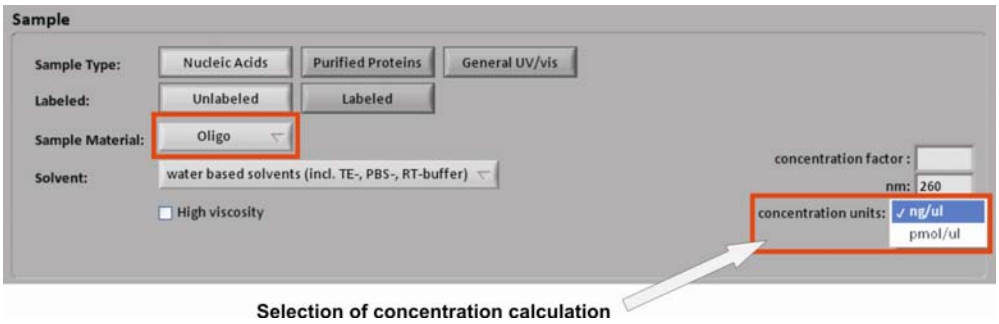
Pressing the 'next' button on the 'Start new measurement' screen will open the next screen where information on sample data and DropPlates96 layout can be entered. The positioning information of samples or blank can be entered manually or can be imported from an excel, text or .csv-file. Both import methods are described in detail in section 4.1.4.



5.1.4 Extra: Quantification of oligonucleotides

Since oligo's have defined sequences, their molecular weight, molar extinction coefficient and the concentration factor specific to that sequence can be calculated and used in the concentration calculation. With the DropSense96, it is posible to measure different oligo's in the same experiment using their specific sequence information. The mode of action to quantify oligonucleotides is slightly different from that of other DNA/RNA quantifications.

Continue on the 'Start new measurement' screen. Fill in this sheet as described in section 5.1.2. Choose as 'sample type': Nucleic Acids and 'sample material': Oligo. An aditional selection between concentration calculation in µg/µl or pmol/µl can be done on the right side of the 'Start new measurement' screen. This selection will also be used in the data analysis section.

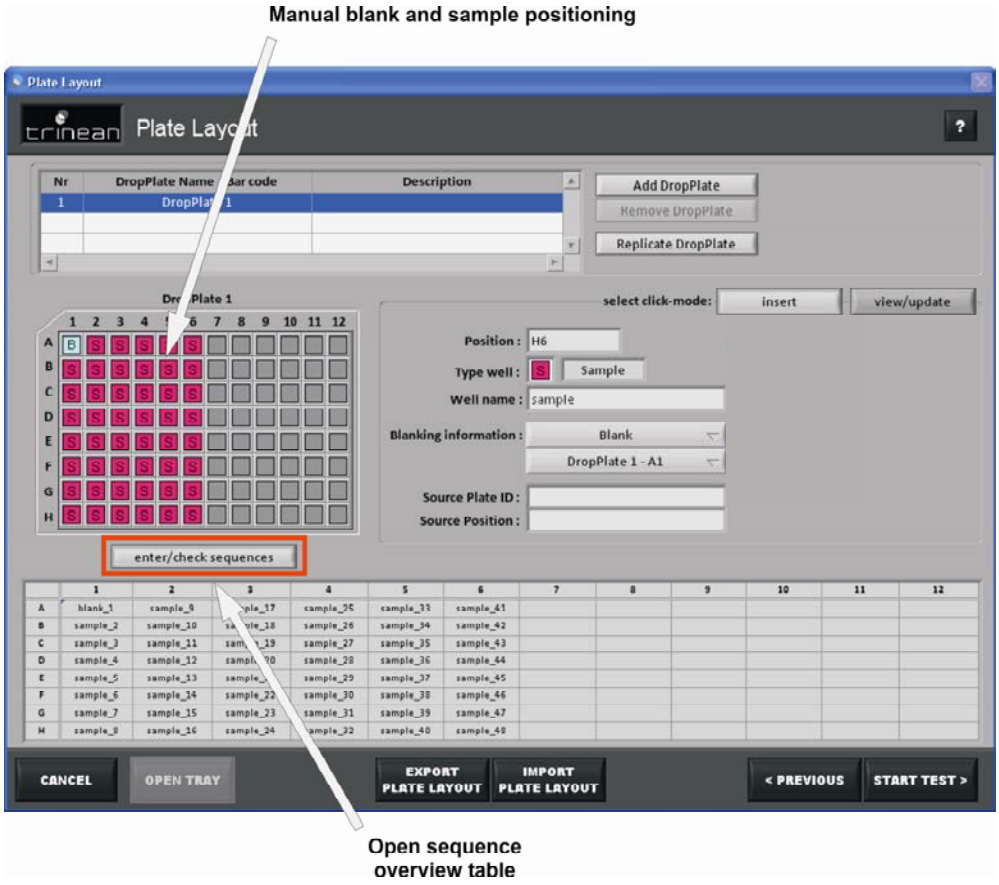


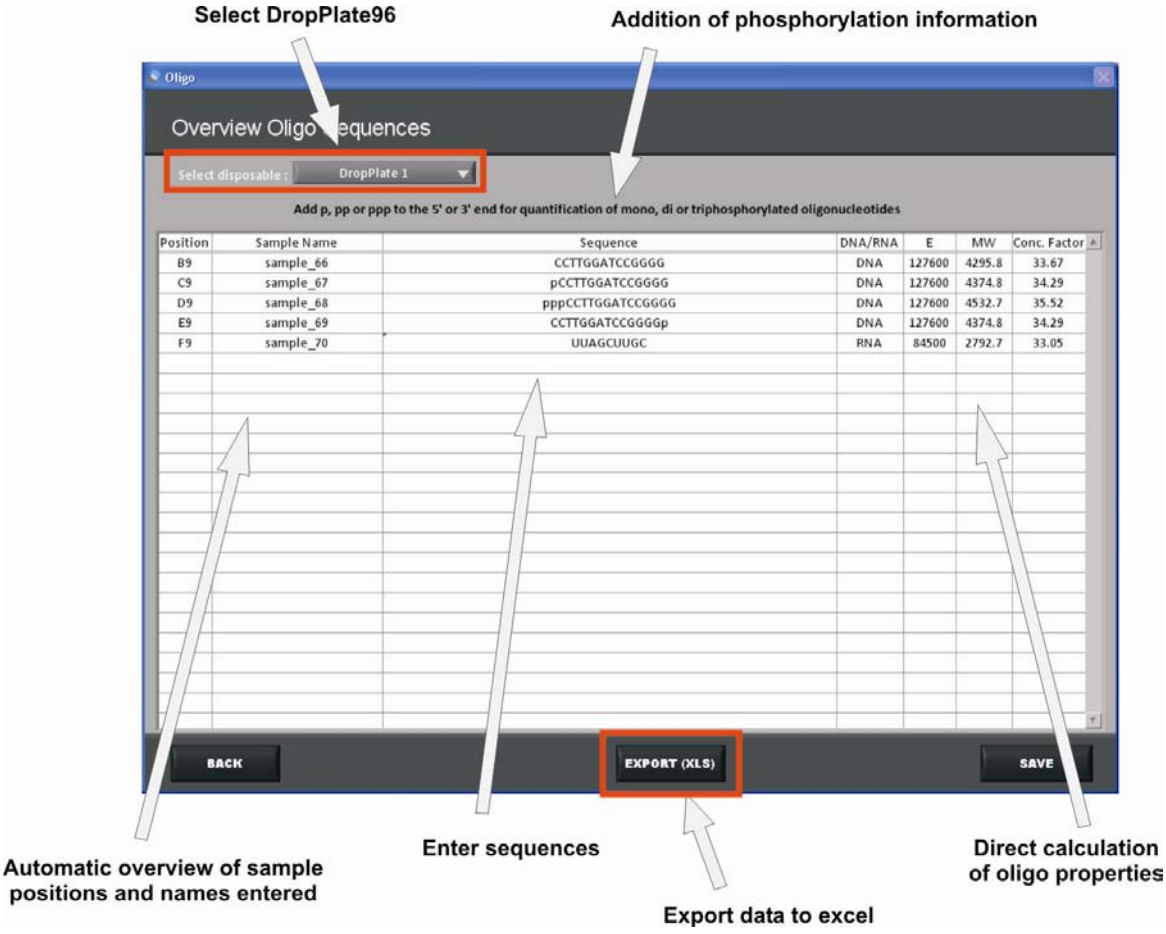
Selection of concentration calculation

Other options like solvent, labels and background correction can be selected as for other nucleic acid samples (see 5.1.2). No information about the concentration factor on the right side is shown yet as this information will be calculated using the oligo sequence. Press the 'next' button to continue on the 'Plate layout' screen. When entering the blank and sample positions manually on the plate layout screen, proceed as described in section 5.1.3, and then press the 'Enter/check sequences' button.

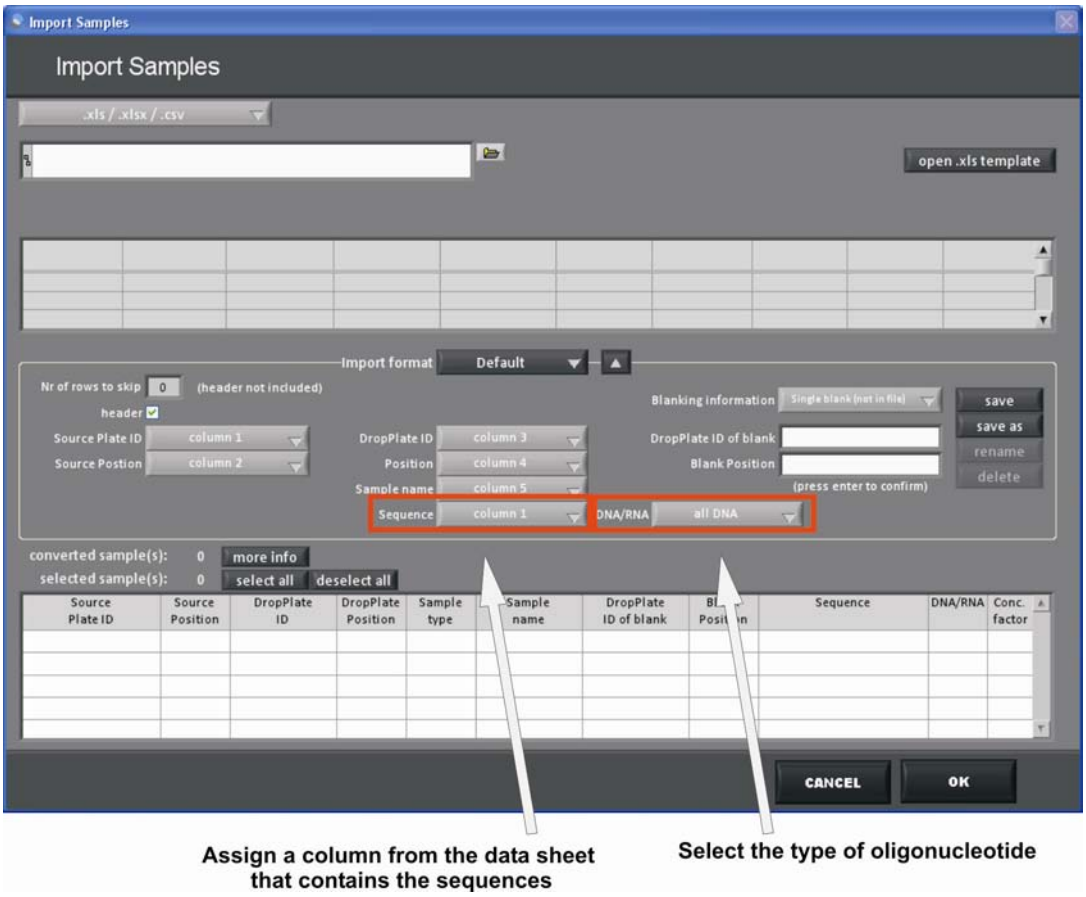
Now, a table will appear with a overview of the selected wells. Type in the proper oligonucleotide sequences or copy/past them from an excel table. The software will calculate the nessecary properties like the exctinction coefficient, the molecular weight and the concentration factor. All the information can be saved in excel by pressing the 'export excel' button at the bottom of the screen. Phosporylations at both the 5' and 3' end can also be included by adding the right number of the letter 'p' to the sequence. Press 'Save' to return to the 'Plate layout' screen.

When using the 'Replicates' function (see section 4.1.4.1), entering the sequence of one sample will automatically lead to the fill in of the sequence and data its replicates.


Manual blank and sample positioning
Plate Layout
Add DropPlate
Remove DropPlate
Replicate DropPlate
select click-mode:
insert
view/update
Position :
Type well :
Sample
Well name :
sample
Blanking information :
Blank
Source Plate ID :
Source Position :
enter/check sequences
CANCEL
OPEN TRAY
EXPORT PLATE LAYOUT
IMPORT PLATE LAYOUT
< PREVIOUS
START TEST >
Open sequence overview table


Select DropPlate96
Addition of phosphorylation information
Overview Oligo Sequences
Select disposable :
DropPlate 1
Add p, pp or ppp to the 5' or 3' end for quantification of mono, di or triphosphorylated oligonucleotides
Position
Sample Name
Sequence
DNA/RNA
E
MW
Conc. Factor
B9 sample_66 CCTTGGATCCGGGG DNA 127600 4295.8 33.67
C9 sample_67 pCCTTGGATCCGGGG DNA 127600 4374.8 34.29
D9 sample_68 pppCCTTGGATCCGGGG DNA 127600 4532.7 35.52
E9 sample_69 CCTTGGATCCGGGGp DNA 127600 4374.8 34.29
F9 sample_70 UUAGCUUGC RNA 84500 2792.7 33.05
BACK
EXPORT (XLS)
SAVE
Automatic overview of sample positions and names entered
Enter sequences
Direct calculation of oligo properties
Export data to excel

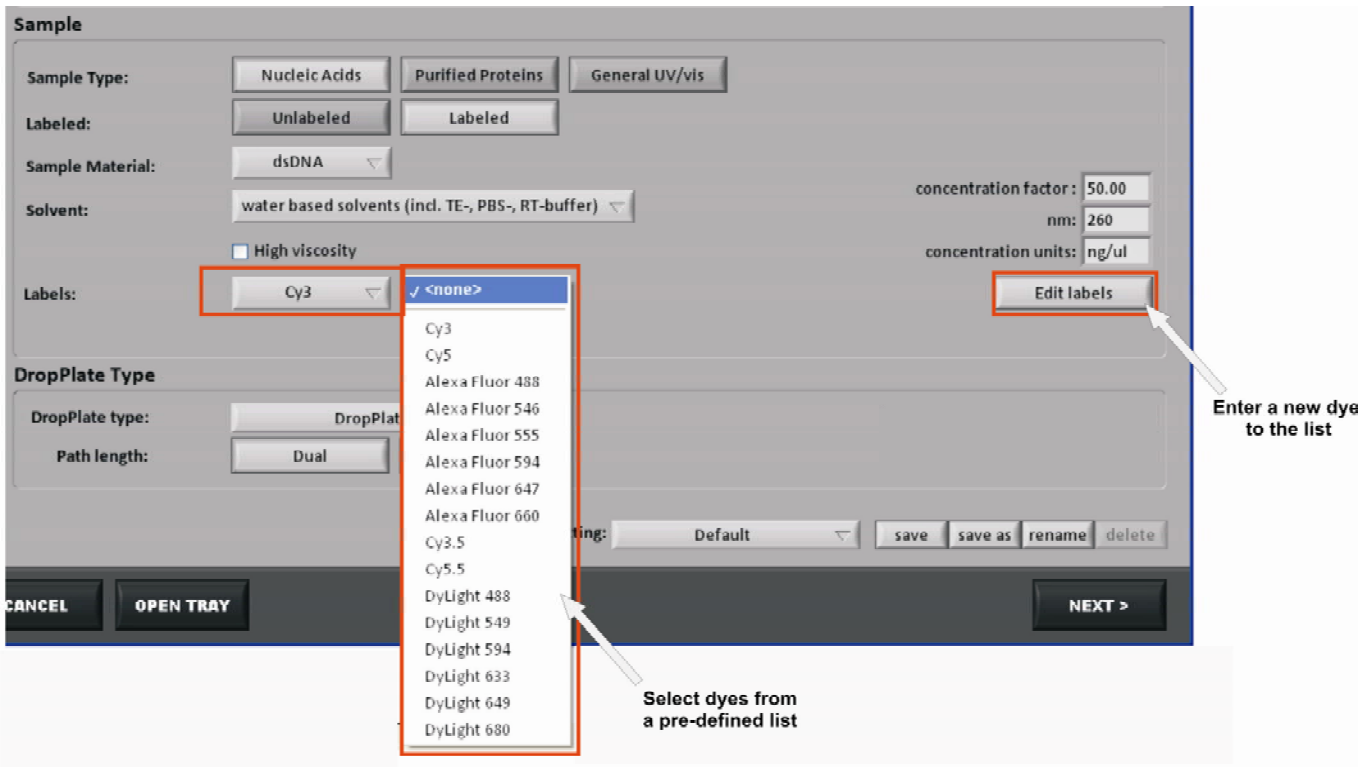
When using the import option (section 4.1.4.2), two extra columns need to be selected so the software can import all the oligo sequences from the used data. One column containing the sequences need to be selected. As for the manual sequuence input, phosphorylion of oligonucleotides can be entered using the right number of the letter ‘p’ added to the 5’ or 3’ end of the sequence. For correct calculation of the extinction coefficient, a selection of nucleic acid type of the oligo has to be made by selecting DNA, RNA or assignment of a column with that information (the column can contain both DNA or RNA). Upon returning to the plate layout screen, an overview of these sequences and properties can be shown by clicking on the ‘enter/check sequences’ button.



Press ‘start test’ to load the first DropPlate96 on the DropSense96 and measurements will start.

5.1.5 Extra: quantification of fluorescent labeled nucleic acids

Measuring the labeling efficiency of fluorescent-tagged probes before micro-array hybridization eliminates potentially flawed samples and improves experiment effectiveness. The full spectrum analysis with the DropSense96 allows measurement of nucleic acid absorption at 260 nm while detecting the incorporated fluorescent dye at their absorption peak. In a single measurement, the DropQuant software calculates the nucleic acid concentration is expressed in µg/µl and the dye concentrations in pmol/µl.

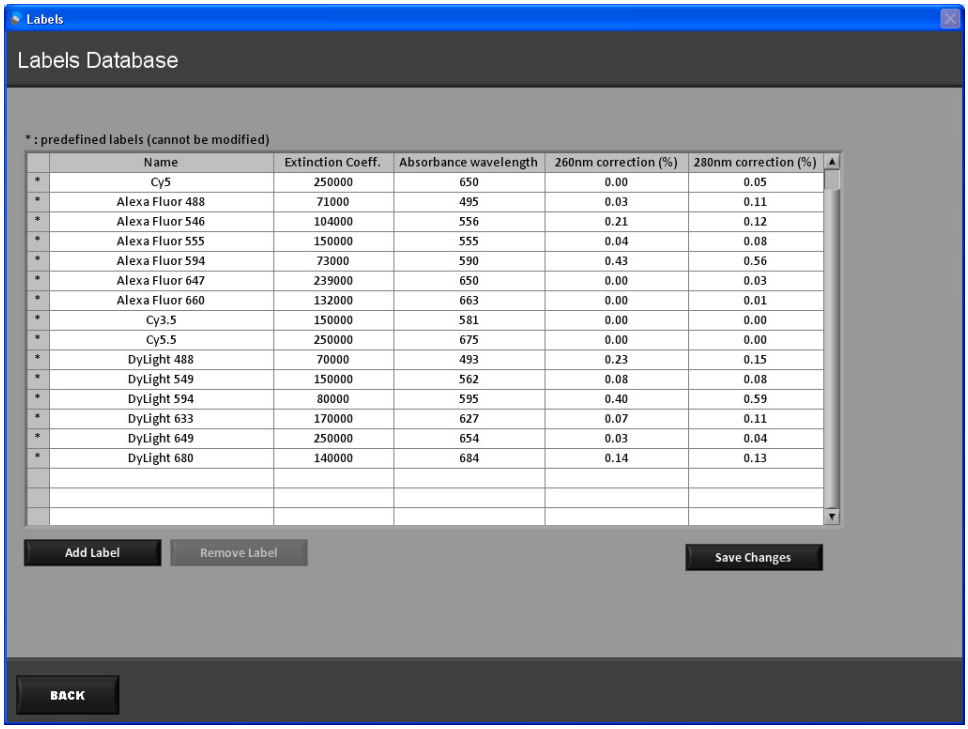


Continue on the 'Start new measurement' screen. Fill in this sheet as described in section 4.1.2. Choose as 'sample type': Nucleic Acids and choose the option 'Labeled'. Other options like solvent can be selected as for other nucleic acid samples (see 4.1.2).

With selecting the option 'Labeled', two drop down lists appear, each containing a wide range of commercially available fluorescent dyes. Use these drop-down lists to select the appropriate dye (or dyes). If the nucleic acids have been labeled with only one dye, choose None as the dye type for the second list.

To enter a new dye, select the 'Edit labels' button on the right side of the screen. This will open a new screen for manual entry of the dye information. Press the 'Add label' button to enter the name and properties given by the manufacturer, like the appropriate correction

factors, can be entered. The 260 nm correction will be utilized for nucleic acid concentration calculation when the respective dye is selected. To remove a dye, select the dye and press the 'Remove label' button. Pre-defined dyes, identified by a '*' next to the label name may not be edited or deleted.



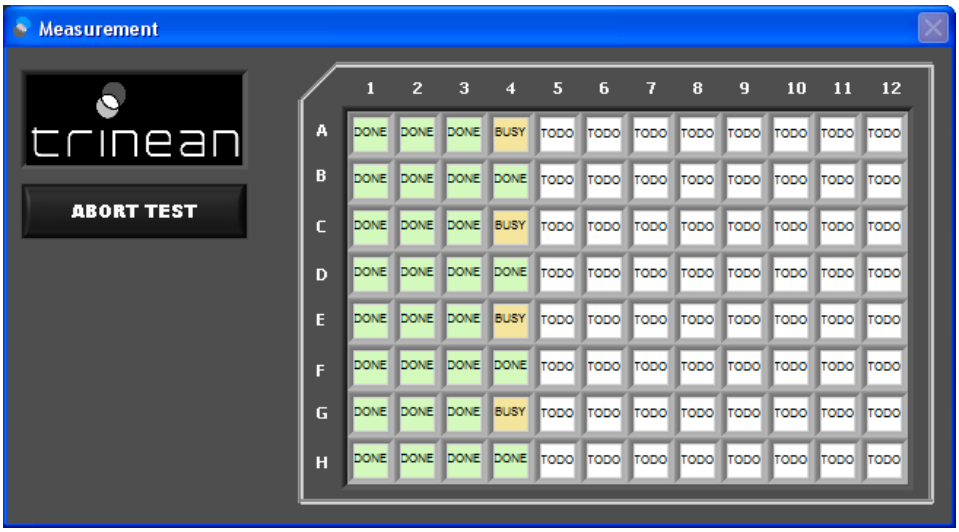
Press the 'Save changes' button and then 'Back' to return to the 'New measurement' page.

5.1.6 Start measurement

Press the ‘Start Test’ button on the task bar of the ‘Plate layout’ screen. This will open the microplate tray of the connected DropSense96 and the following screen will appear:

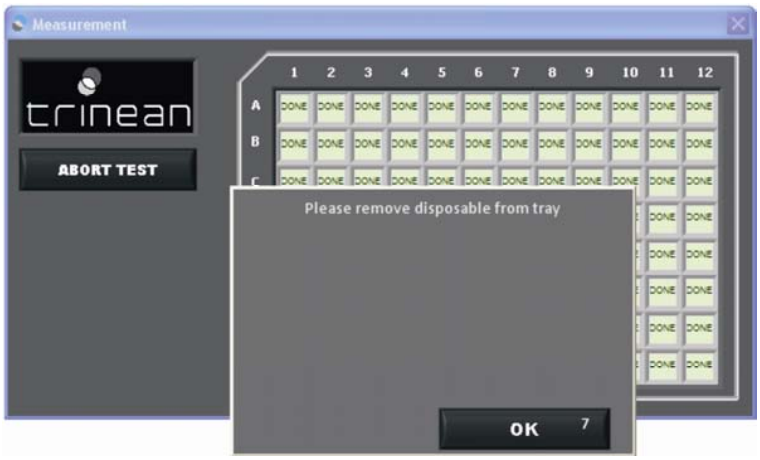
Load the first DropPlate (DropPlate1) on the tray and press ‘OK’. The tray will be pulled in and the measurements will start. Press ‘Cancel’ if the measurement should not take place. The software will return to the main menu.

The progress of a measurement can be observed in the small measurement screen, showing wells that have been measured (indicated by a green color and ‘done’), wells being measured (orange and indicating ‘busy’) and wells that are still waiting to be measured (white color and indicating ‘To do’).

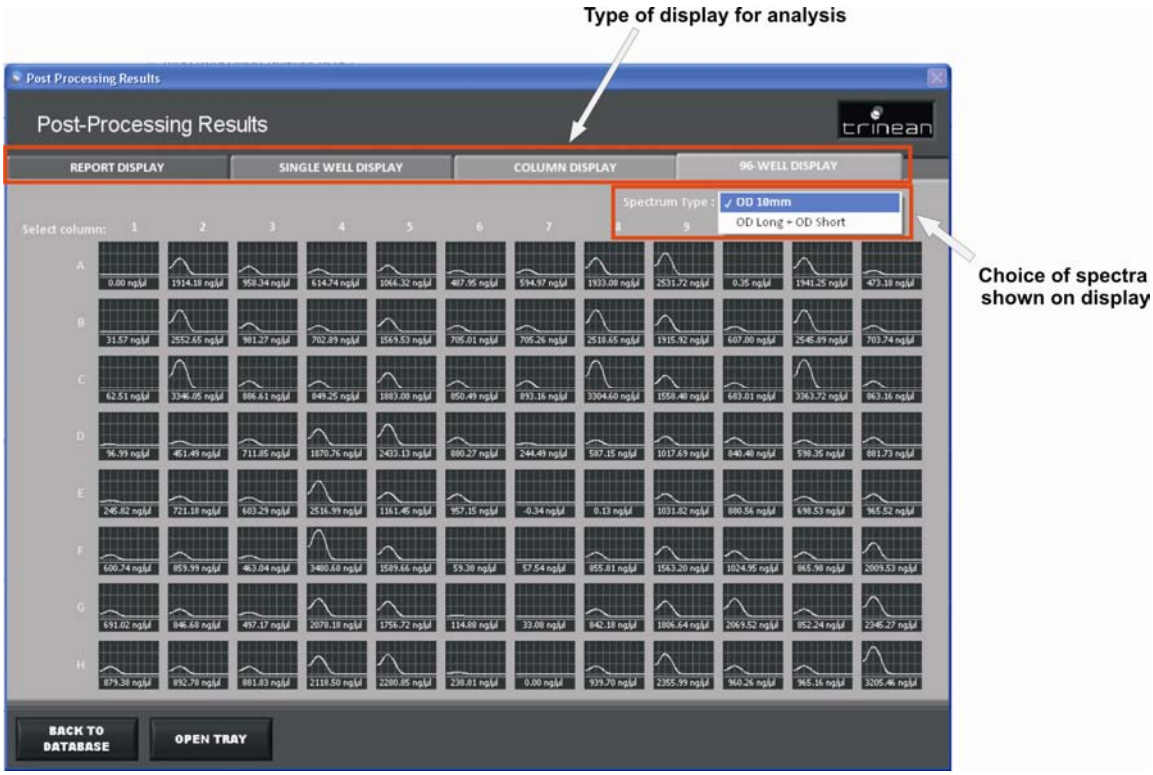

Measurement
trinean
ABORT TEST
1 2 3 4 5 6 7 8 9 10 11 12
A DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
B DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
C DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
D DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
E DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
F DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
G DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
H DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO

5.1.7 Data analysis

When the full measurement is done, following screen appears:

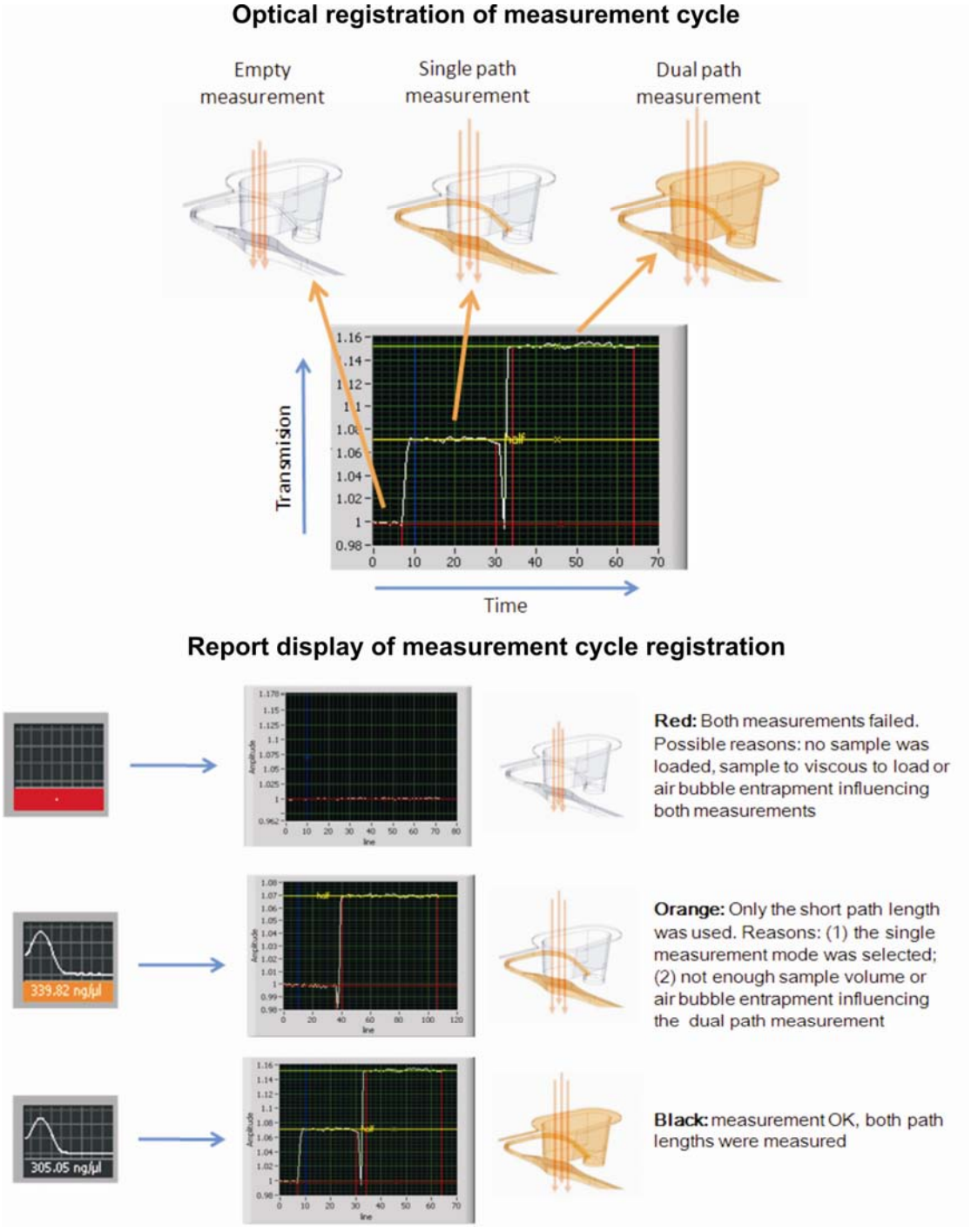


You can take the DropPlate96 of the microplate tray and press 'OK'. Now the software will generate the full spectral scan of all samples and calculate the concentration of the nucleic acid sample. For safety reasons, the microplate tray with the DropPlate96 will be pulled in automatically after 30 seconds and the measurements will be displayed. Several display options can be chosen. As default, an 96well overview of the measurements is given. Detailed analysis with setting options can be done in the 'single well display', as explained in section 5.1.7.3.



5.1.7.1 96well display:

Gives an overview of all spectra and the concentration of the nucleic acid calculated from A260 (See example above). In the standard mode, all spectra are shown normalized to a 10mm path. For a DropPlate-D measurement, a combined spectral view of both path lengths can be shown by selecting 'OD long + OD short' in the 'Spectral Type' button. Detailed analysis of one sample can be achieved by clicking on the sample spectrum, opening a detailed figure of this spectrum in the single well display.

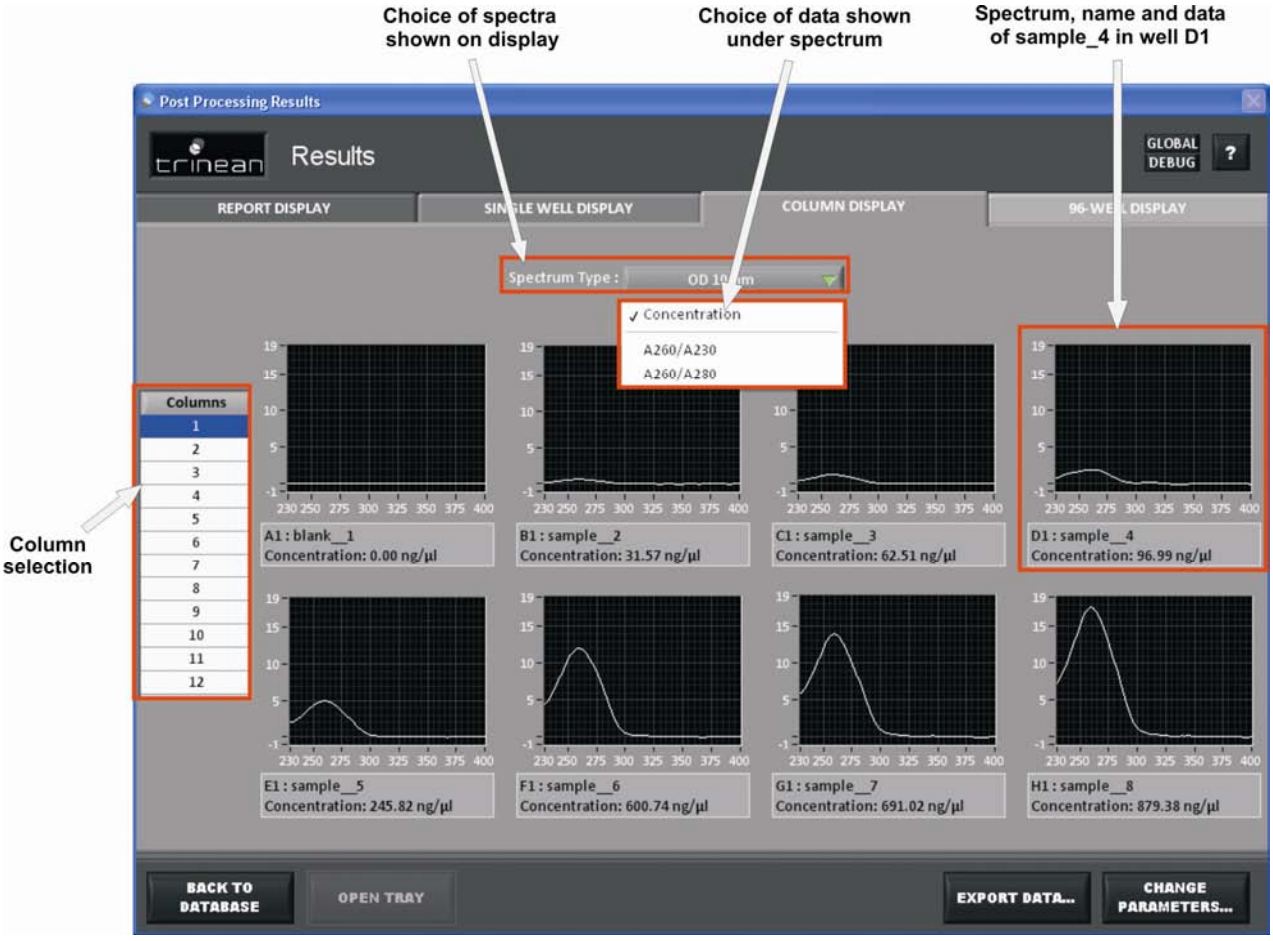

Optical registration of measurement cycle
Empty measurement
Single path measurement
Dual path measurement
Transmision
Time
Report display of measurement cycle registration
339.82 ng/µl
305.05 ng/µl
Red: Both measurements failed. Possible reasons: no sample was loaded, sample to viscous to load or air bubble entrapment influencing both measurements
Orange: Only the short path length was used. Reasons: (1) the single measurement mode was selected; (2) not enough sample volume or air bubble entrapment influencing the dual path measurement
Black: measurement OK, both path lengths were measured

When using **DropPlate-D** consumables, the software will automatically select one of the dual path lenght measurement and normalizes the data to a 1.0 cm (10.0 mm) path. As a general rule: the 1mm pathlenght is used up to OD 1 (OD 10 when normalized t a 1 cm path). Above 1 OD at 1 mm, the concentration is calculated with the 0.2 mm path absorbance measurement.

During the measurement cycle, the transport of the sample through the microfluidic structure is monitored by continuous spectral measuring. This way, errors occuring during the process can be detected and displayed on the screen. This allows a thourough and robust analysis of all measurements. A color legend is used as illustrated on the previous page.

When using **DropPlate-S** consumables, the software will normalize the single cuvette measurement (0.5mm path) to a standard 10.0 mm path. Similar color legends are used as with the DropPlate-D. A black background indicates a good measurement, while a red color inducates a failed measurement. As the DropPlate-S only contains one micro-cuvette, no orange color legend is used.

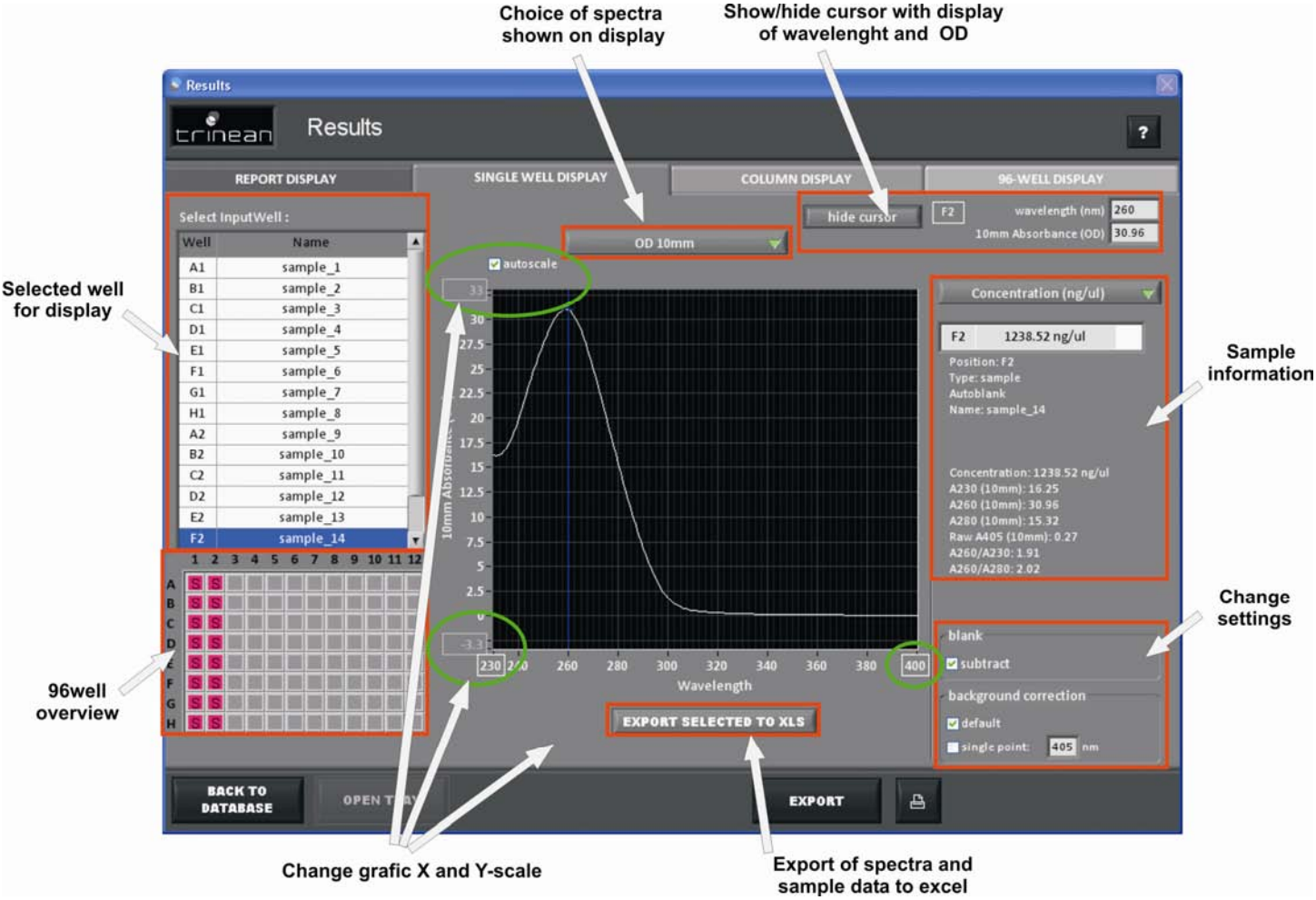
5.1.7.2 Column display:



Gives an overview of all measurements per column. Clicking on another column opens all spectra of this column. Underneath every spectrum, the sample name and data are displayed. A data selection can be done using the scroll button above the spectra. A choice can be made between 'concentration', 'A260/A230' or 'A260/A280' ratio. The view of the spectra can be changed from 'OD 10mm' to the combined view 'OD long + OD short' using the 'Spectrum Type' button.

5.1.7.3 Single well display

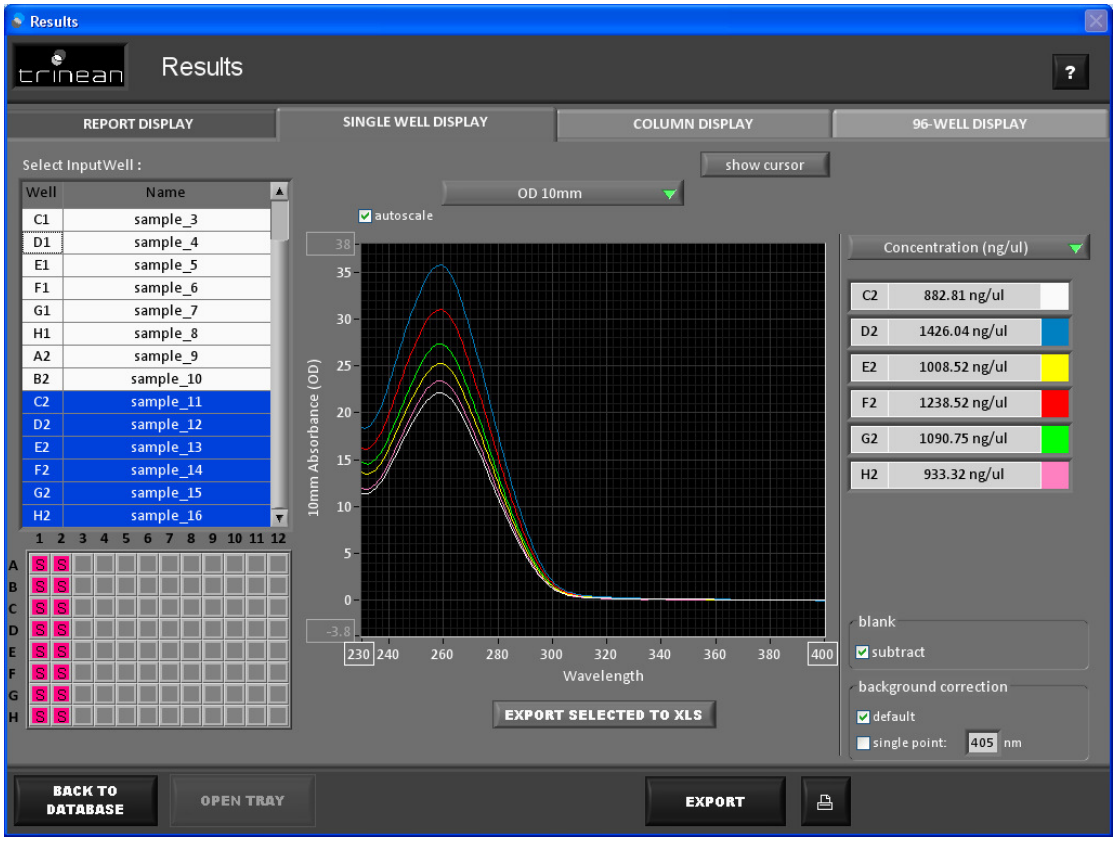
Gives a detailed view of a sample spectrum and all additional sample data next to the spectrum. A sample can be viewed by clicking on the name of the well or sample in the 'select input well' table or by clicking on a well in the 96well overview.



Extra tools are included for detailed analysis and comparison of the samples:

- **Hide/Show cursor**: with this cursor, a specific wavelength can be selected by dragging the blue cursor. The selected wavelength and its OD value (10 mm) is displayed

- **Path length selection**: Above the spectral view, a choice can be made between 'OD 10mm' or 'OD 1.0 mm + OD 0.2mm' (for a DropPlate-D measurement) or 'OD 0.5mm' (for a DropPlate-S measurement). Note that the Y-axis scale will automatically adjust to the proper OD values. When selecting 'OD 1.0 mm + OD 0.2mm', the 1.0mm OD spectra will be shown with a full line, while the 0.2mm OD spectra will be displayed using a dotted line.
- **Overlay spectra:** when pressing the CTRL button on the computer keyboard, select two or more samples from the 'select input well' table to overlay the selected spectra. When using the 'shift' button and selecting two samples, all samples between the selected will also be displayed on the screen. (Underneath, RNA samples are shown)



Additional tools are included to change the analysis settings of the measurements. Changes made within these tools (explianed underneath) stay fixed within the other data analysis screens and the export options.

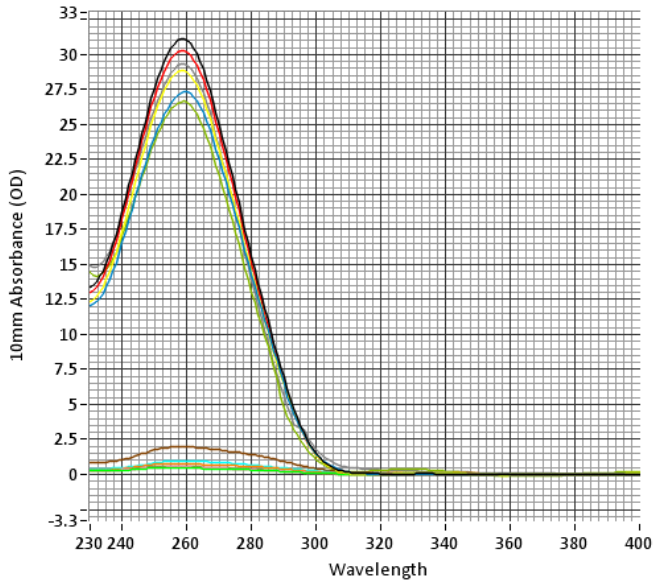
- **Change scale:** Both the scales of the X-axis and the Y-axis can be altered for a detailed view by changing the highlighted wavelength at the beginning and end of the scale. The setting 'autoscale' has to be turned off when changing Y-axis. Note that the report option 'All absorbance values (10mm)' will show all the absorbance values within the selected wavelength range of the X-axis. So manual changing of the X-axis scale will change the info in the report.
- **Blank subtraction**: When using a blank reference sample, it is automatically subtracted from the sample absorbance before concentration calculation. This feature can be turned off so the spectral shape and concentration calculation is shown without blank compensation. In this mode, the shape of the blank spectrum itself can be analyzed. When the blank subtraction is turned off, all other screens and the report section will generate data without blank subtraction.
- **Background correction**: the background correction method can be selected in the 'start new measurement' sheet (section 5.1.2). The background information is derived from the RAW OD spectrum measured. In the default setting, a linear fit through the 400-600 nm region of the transmission spectrum is subtracted from the full spectrum, while in the single point mode, the background level of a wavelength of choice is subtracted from the full spectrum. The manual selection on the 'single well display' offers the option to chose between the 'default' method, a single wavelength of choice or no background correction. This allows to verify if the background is not near zero as indicator for turbidity or presence of debris. Note that changes in the background correction will be used in the other screens and report section. In the report section, the RAW OD value of the wavelength shown in the single point background correction (for instance RAW A405 (10 mm)) can be included in the report table to export.

The 'single well display' offers a '**quick export to XLS**' function located underneath the spectral view. This function is limited to an export to Excel, showing all the spectra selected on the 'single well display' and a fixed selection of columns with all necessary data from the selected samples.

An example is shown on the next page

trinean

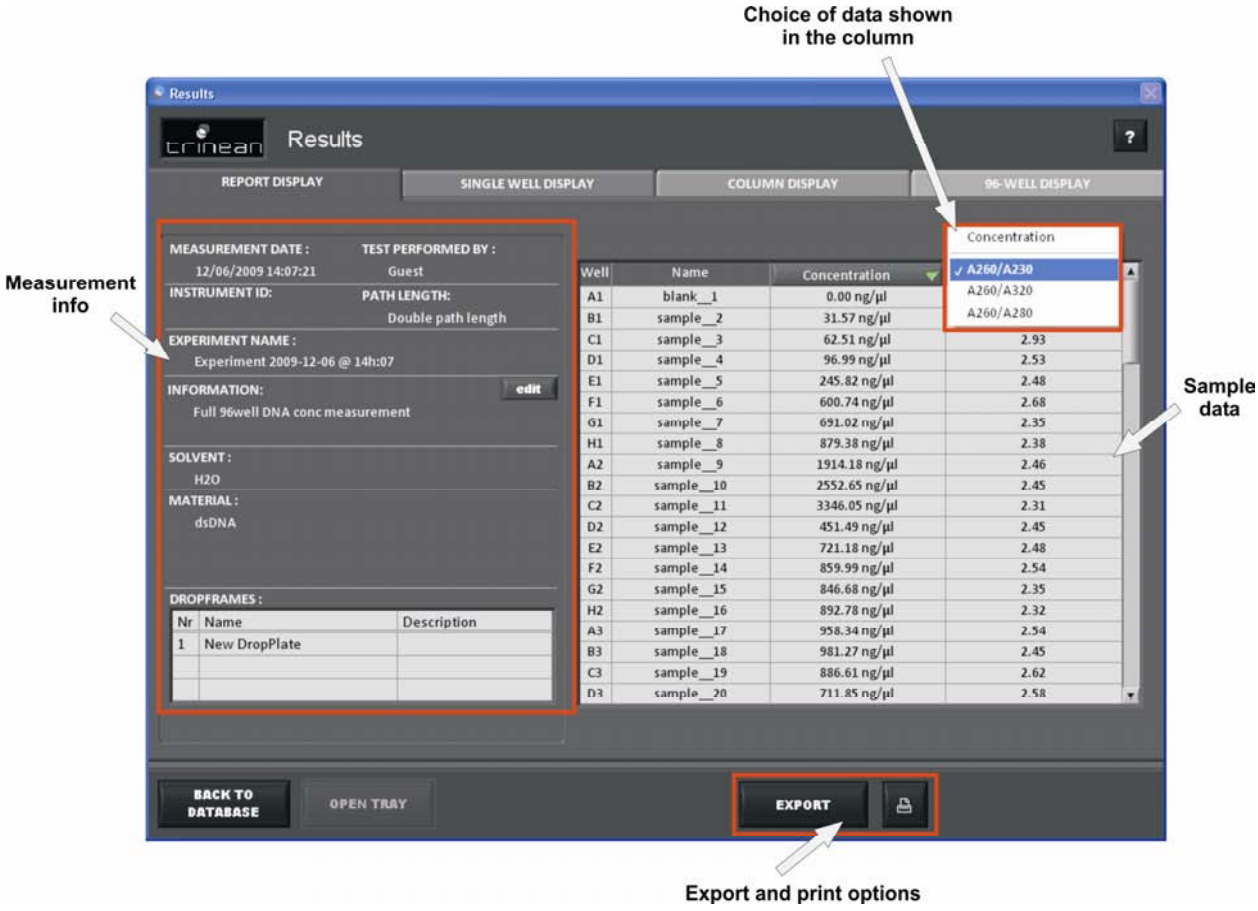
DropSense REPORT



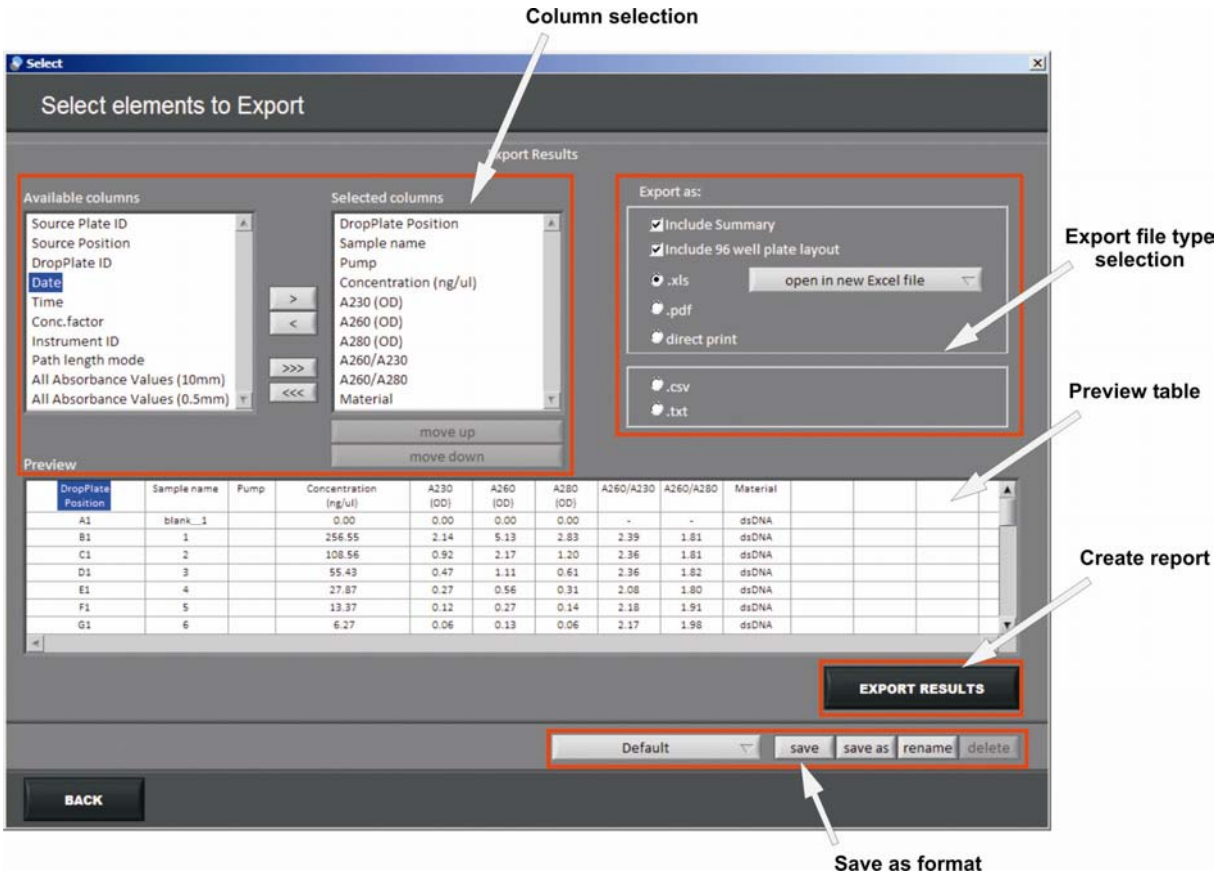
	DropPlate ID	DropPlate Position	Sample name	Pump	Concentration (ng/ul)	A230 (10mm)	A260 (10mm)	A280 (10mm)	Raw A405 (10mm)	A260/A230	A260/A280
	DropPlate 1	G1	sample_7		1240.89	13.42	31.02	15.54	-0.04	2.31	2
	DropPlate 1	H1	sample_8		1092.33	12.06	27.31	14.26	-0.07	2.26	1.91
	DropPlate 1	A2	sample_9		1148.49	12.39	28.71	14.47	-0.17	2.32	1.98
	DropPlate 1	B2	sample_10		1206.4	13.01	30.16	15.11	-0.05	2.32	2
	DropPlate 1	C2	sample_11		18.6	0.25	0.47	0.33	-0.06	1.88	1.42
	DropPlate 1	D2	sample_12		21.07	0.29	0.53	0.35	-0.06	1.83	1.5
	DropPlate 1	E2	sample_13		22.78	0.26	0.57	0.38	-0.08	2.18	1.5
	DropPlate 1	F2	sample_14		30.11	0.31	0.75	0.57	-0.15	2.4	1.32
	DropPlate 1	G2	sample_15		38.37	0.41	0.96	0.67	-0.13	2.36	1.43
	DropPlate 1	H2	sample_16		78.92	0.86	1.97	1.42	-0.05	2.3	1.39
	DropPlate 1	B3	sample_18		1062.32	14.42	26.56	13.05	0.6	1.84	2.04
	DropPlate 1	C3	sample_19		1170.11	14.9	29.25	13.88	1.69	1.96	2.11

5.1.7.4 Report display

The report section gives an overview of the experiment information and all the measurement data in a table format. The info on the data table can be altered by clicking on the header of the column, concentration, 260/230 or 260/280 ratio's can be chosen. Furthermore, the measurement description can be altered by pressing the 'edit' button. This overview can be exported to several formats like a CSV, txt, excel sheet or pdf file. The data can also be send directly to a printer of choice.



The 'Export' button opens a new screen to create a data report as desired. In the **results export sheet**, a selection of columns can be made to be included in all three report types. The order of the columns can be altered with the 'move up' and 'move down' buttons. A preview of the report table is displayed at the bottom of the screen. The export file type can be selected by clicking on the preferred file type. Furthermore, the information from the summary (experiment description, experiment name, solvent, …) and an overview of the plate layout can also be included by activating the 'include summary' button. The report design can be saved in a format for future use by clicking the 'save as' button.


Column selection
Select
Select elements to Export
Export Results
Available columns
Source Plate ID
Source Position
DropPlate ID
Date
Time
Conc.factor
Instrument ID
Path length mode
All Absorbance Values (10mm)
All Absorbance Values (0.5mm)
>
<
>>>
<<<
Selected columns
DropPlate Position
Sample name
Pump
Concentration (ng/ul)
A230 (OD)
A260 (OD)
A280 (OD)
A260/A230
A260/A280
Material
move up
move down
Export as:
Include Summary
Include 96 well plate layout
.xls
open in new Excel file
.pdf
direct print
.csv
.txt
Export file type selection
Preview table
Preview
DropPlate Position | Sample name | Pump | Concentration (ng/ul) | A230 (OD) | A260 (OD) | A280 (OD) | A260/A230 | A260/A280 | Material
A1 | blank_1 | | 0.00 | 0.00 | 0.00 | 0.00 | - | - | dsDNA
B1 | 1 | | 256.55 | 2.14 | 5.13 | 2.83 | 2.39 | 1.81 | dsDNA
C1 | 2 | | 108.56 | 0.92 | 2.17 | 1.20 | 2.36 | 1.81 | dsDNA
D1 | 3 | | 55.43 | 0.47 | 1.11 | 0.61 | 2.36 | 1.82 | dsDNA
E1 | 4 | | 27.87 | 0.27 | 0.56 | 0.31 | 2.08 | 1.80 | dsDNA
F1 | 5 | | 13.37 | 0.12 | 0.27 | 0.14 | 2.18 | 1.91 | dsDNA
G1 | 6 | | 6.27 | 0.06 | 0.13 | 0.06 | 2.17 | 1.98 | dsDNA
Create report
EXPORT RESULTS
Default
save
save as
rename
delete
BACK
Save as format

5.2 Protein quantification

5.2.1 Theory: Protein concentration and purity determination

Protein concentration, like nucleic acids, can be determined by measuring their UV absorbance at 280nm and calculating the concentration using the extinction coefficient of the protein in the Beer-Lambert equation. This method is suited to quantify purified proteins.

However, the 280nm absorption of proteins depends on the presence of aromatic amino acids (Trp, Tyr and Phe) and Cys-Cys disulfide bonds. Therefore, the UV absorption of proteins varies greatly and depends on the particular amino acid concentration of a protein. In addition, buffer type, ionic strength and pH affect the UV absorption and even pure protein solutions may have different conformations and modifications. When performing protein A280 concentration measurements, the best approach is to empirically derive the extinction coefficient for the protein of interest, or search for published protein extinction coefficients (examples in the Practical Handbook of Biochemistry and Molecular Biology). Alternatively, if the protein sequence of the protein to be measured is known, the theoretical molar extinction coefficient can be calculated using the equation:

$$\varepsilon = 5500(\#Trp)+1490(\#Tyr)+125(\#Cys)$$

Where:

ε = the extinction coefficient

= number of

Trp = Tryptophan, Tyr = Tyrosines, Cys = Cysteines

A very rough protein concentration can be obtained by making the assumption that the protein sample has an extinction coefficient of 1, so 1 OD = 1 mg/ml protein.

In combination with the DropPlate-D consumable, the DropSense96 performs a dual path measurement, with path lengths of 0.2 and 1mm to enable quantification of proteins with a wide concentration range without need for dilution. Using the DropPlate-S with a single micro-cuvette (0.5mm path) on the DropSense96 is ideal for quick analysis of protein samples within a more limited measurement range.

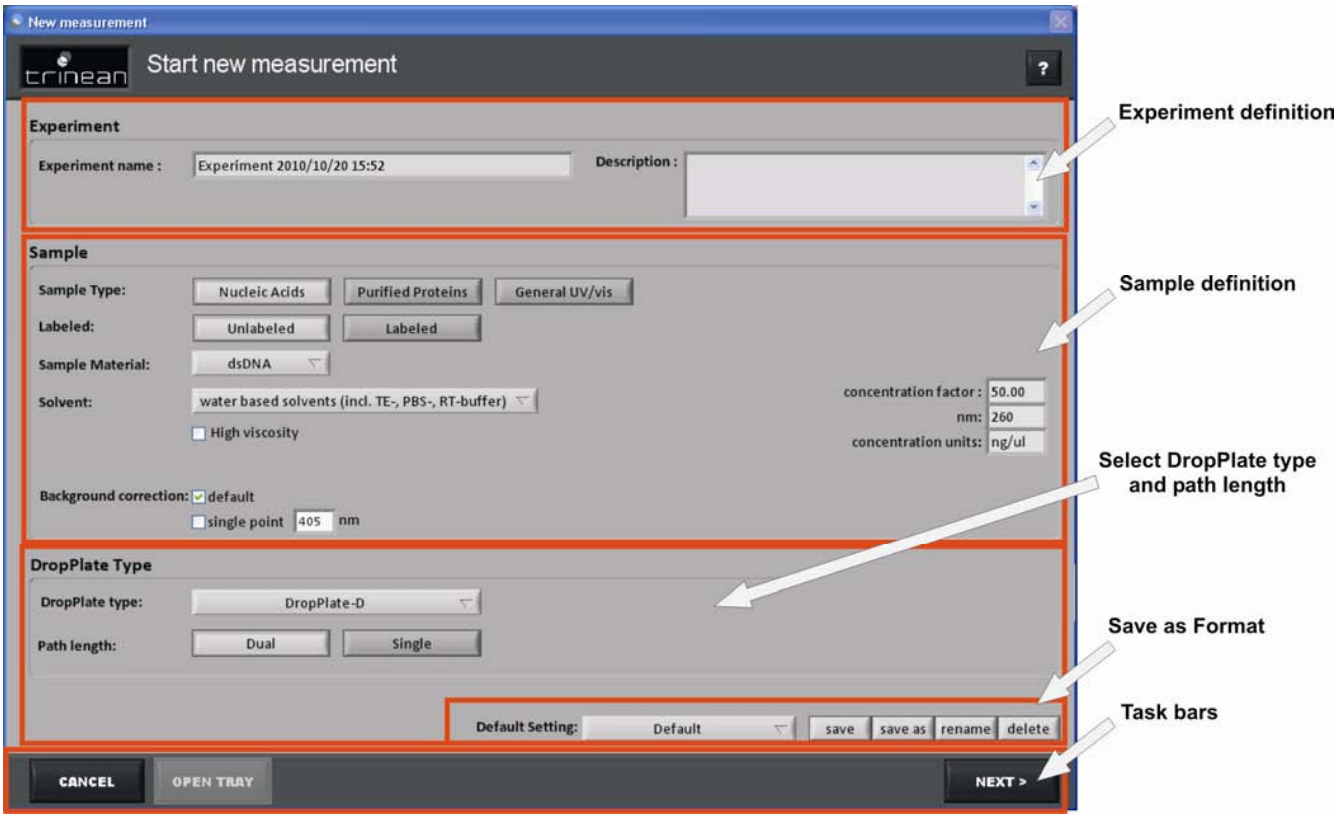
The absorbance data are archived in the database and displayed on the software screen after measurement. In the protein A280 application, the software chooses automatically which path length generates the best absorbance values and normalizes this measurement to a 1.0 cm (10.0 mm) path. These are displayed in the software for further data analysis.

Since the UV absorption of nucleic acids at 280 nm can be as much as 10 times that of a protein, a small percent of nucleic acids in the sample can greatly distort the protein quantification. Therefore, the protein sample purity must be determined using the A260/A280 ratio. A A260/A280 ratio < 1 indicates "pure" protein whereas a higher value indicates nucleic acid contamination.

An alternative method for protein quantification is a colorimetric protein assay, such as a BCA, Bradford, and Lowry assay. These are commonly used for quantification of protein solutions and cell lysates. These types of assays can easily be performed with the DropSense96, using the 'general UV/vis mode' in the software. Quick export of the data to excel allows fast generation of a standard curve and concentration determination.

5.2.2 Starting a protein concentration measurement

Pressing the 'new measurement' button on the main menu opens following screen:



All options on this screen are described in detail in section 4.1.2.

For measurement of proteins, select the proper preferences in the sample definition box:

Sample Type:

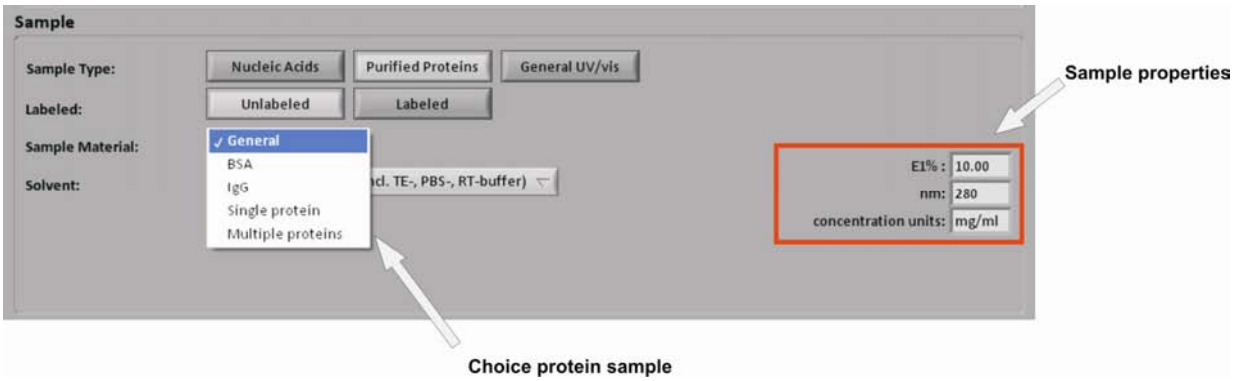
Select 'Purified proteins' (the button turn light gray upon selecting).

Labeled:

Make a choice between 'Unlabeled' for general protein quantification or 'Labeled' when a fluorescent label is present and the concentration needs to be determined.

Sample material:

Select the type of protein in this experiment. Four sample types are available for purified protein analysis by selecting from the drop down list adjacent to 'Sample material'. For every selection, the sample properties used for the concentration calculation are shown on the right side.



The default option 'General' is a reference setting based on the assumption that the protein solution has an extinction coefficient of 1, so 1 OD = 1 mg/ml protein. So a 1% (10mg/ml) protein solution will have an A280 of 10 (for a 10mm path length). This option can be used if no extinction coefficient information exists for the protein sample and a rough estimate of protein concentration is needed.

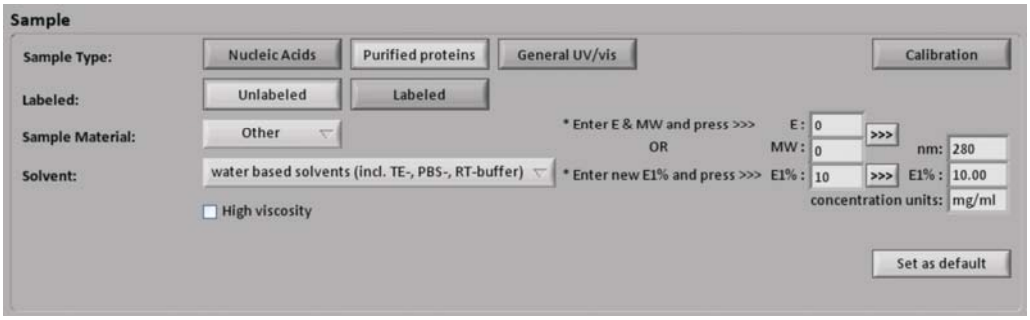
Two protein references are included: **Bovine serum Albumin (BSA)** and **Human Immunoglobulin G (IgG)**. For BSA, a protein sample concentration is calculated using the mass extinction coefficient of 6.67 at 280 nm for a 1% (10 mg/ml) BSA solution. For IgG, a protein sample concentration is calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.

The option 'Other' lets the user enter the **extinction values of a single protein**. Two options can be selected:

- Enter the molar extinction coefficient ($M^{-1}.cm^{-1}$) and molecular weight (MW) in Daltons (Da) of the protein solution. The appropriate ε and the MW (Da) should be entered

and a software calculation of the extinction coefficient for a 10mg/ml solution (E1%) is performed and used to calculate the protein concentration.

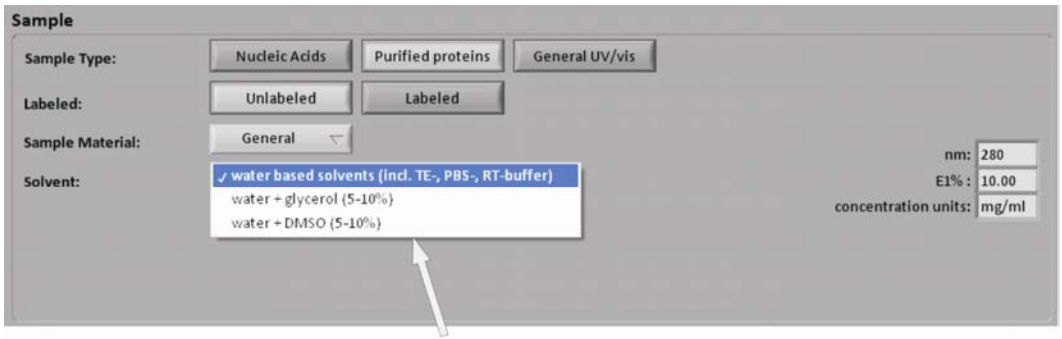
- Enter the mass extinction coefficient ($L.g^{-1}.cm^{-1}$) for a 10mg/ml (1%) protein solution. The appropriate extinction coefficient (E1%) should be entered before the measurement.



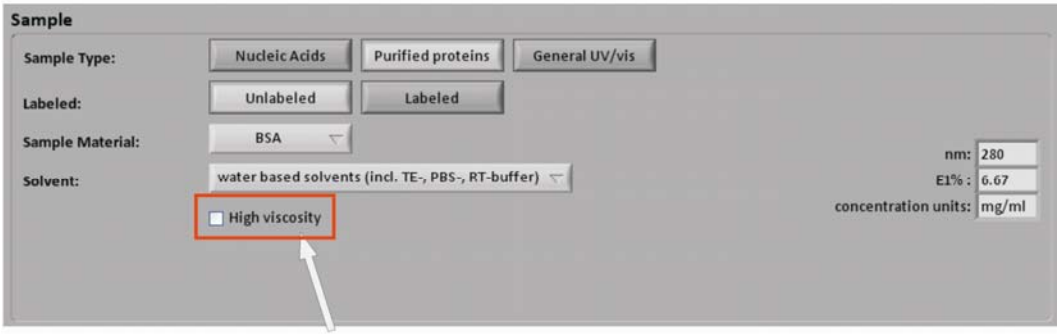
Finally, when measuring a DropPlate96 containing **several proteins with different extinction coefficients**, the multiple proteins option has to be selected. For each protein, the appropriate extinction coefficient (E1%) can be entered for precise concentration determination (see 5.2.4).

Solvent:

Select the solvent used for the protein samples to be measured. This solvent information is used by the DropSense96 to fine-tune the pressures used by the vacuum pumps guaranteeing precise and controlled filling of the microfluidic structures. All water-based



Choice solvent



Extra button for high viscous samples

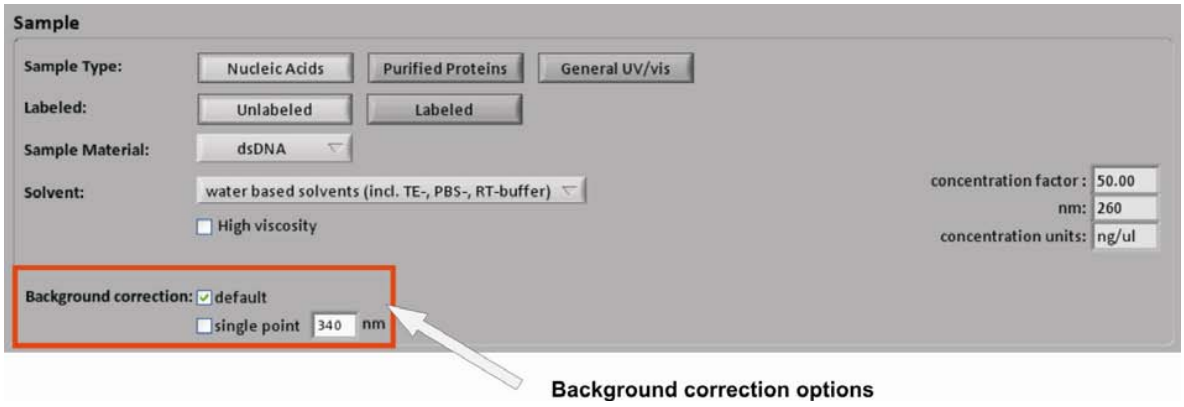
solvents containing low amounts of salts and buffers are grouped in one option since these substances don't affect the pumping properties.

An extra button for highly viscous samples can be used, so the pumps will work at higher pressure and longer duration to the measuring cuvettes on the DropPlates correctly. Viscous protein samples should be handled with care as high viscosity can hamper both precise pipetting of the samples and transport of the sample in the microfluidic DropPlate16.

When the sample is still too viscous, the measurement can fail. This will be indicated by an orange or red color indication when the results are shown (see further).
Furthermore, proteins can denaturate, precipitate or aggregate, leading to non-homogeneous samples.

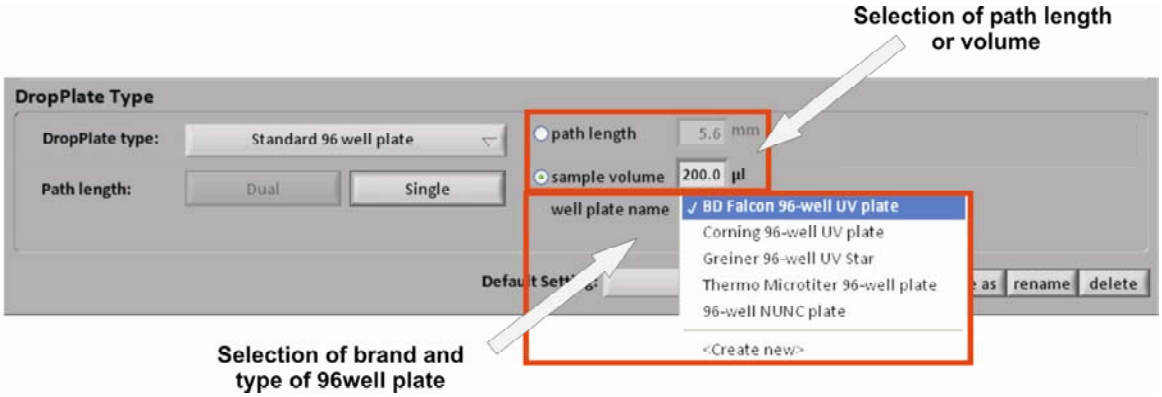
Background correction:

A choice can be made between two types of background corrections. The default background correction determines a linear fit through the 400-600nm part of the spectrum and subtracts this background curve from the full OD range. The second option is to choose a single wavelength (most common used wavelengths by 'classic' spectrophotometers are 320, 340 and 405 nm). The absorbance value of that wavelength will be subtracted from all wavelength data, so the full spectrum will be set so that the background wavelength of choice will be zero. During the data analysis, the background correction can be altered again or turned off for detailed analysis.



DropPlate selection

Depending on the application or sample concentration range, a choice of DropPlate-D or DropPlate-S can be made. Both DropPlates are explained in detail in section 3.1. Alternatively, a standard 96well microtiter plate can be used. This selection requires additional information like path length or sample volume combined with plate brand and type in order to calculate the correct path length. Additional plate brands and types can be entered by selecting '*Create new*' from the drop down list.



Path length selection

When using **DropPlate-D** disposables, the user can select between a single or dual path length measurement (see 3.1.2). The single measurement requires 1 µl of sample to perform a single pumping step and a small chamber measurement for high concentration sample analysis (2-110 OD, 10mm equivalent absorbance). The dual measurement, requiring 3 µl of sample, uses both measurement chambers for the analysis creating the large concentration measurement range (0.05-110 OD, 10mm equivalent absorbance).

When using a **DropPlate-S**, only a single measurement is possible. This consumable requires 2 µl of sample and performs a single measurement with a measurement range of 0.05-44 OD, 10mm equivalent absorbance.

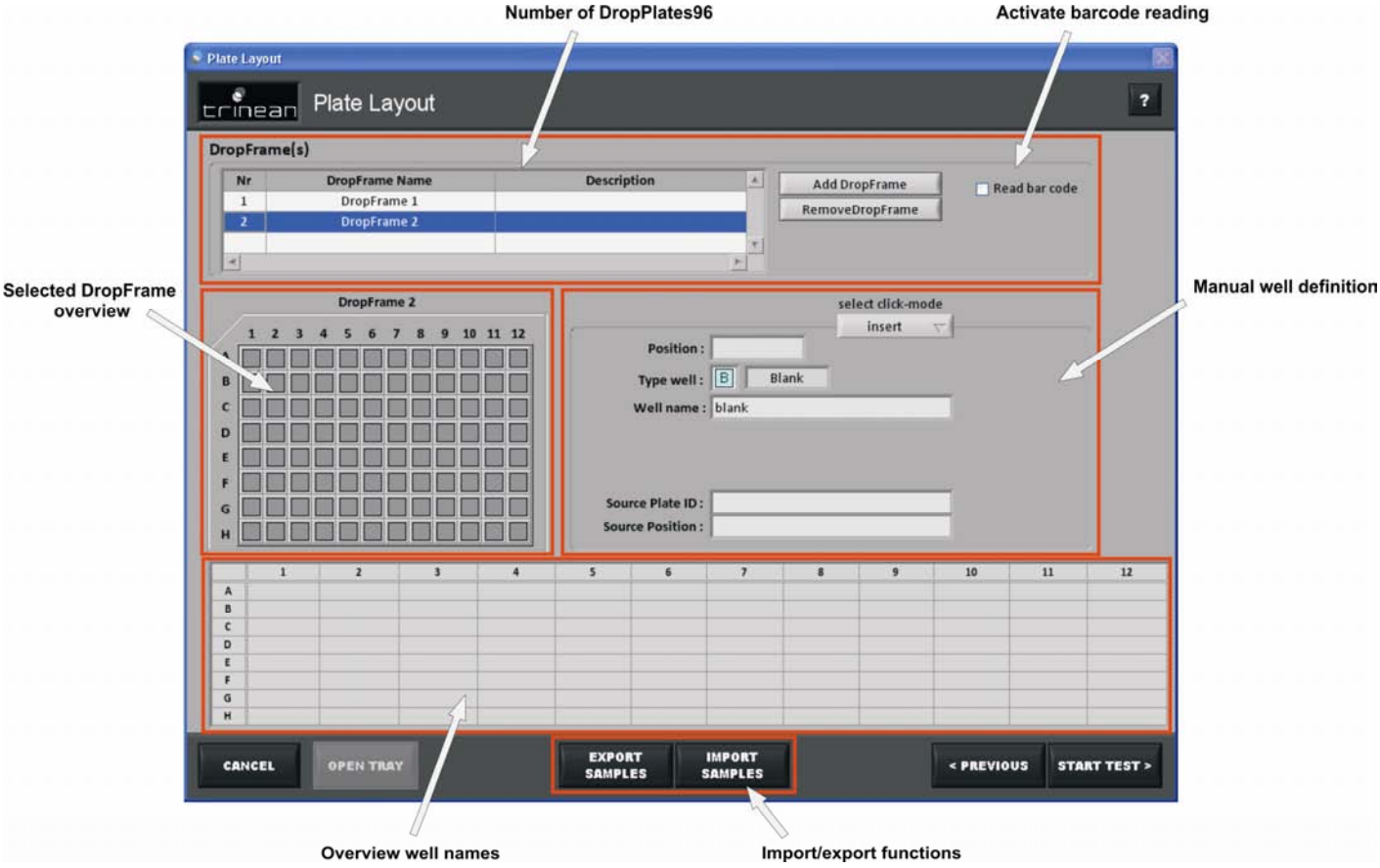
Save as a format:

When all the above selections have been made, the user can save these in a new format for future use. These formats are account dependent. Only the lab manager account has the possibility to make a format that is available for all users. The formats can be deleted, renamed, …

When all sample properties are entered, press the 'next' button on the tast bar below

5.2.3 DropPlate96 sample layout

Pressing the 'next' button on the 'Start new measurement' screen will open the next screen where information on sample data and DropPlates96 layout can be entered. The positioning information of samples or blank can be entered manually or can be imported from an excel, txt or .csv-file. Both import methods are described in detail in section 4.1.4.

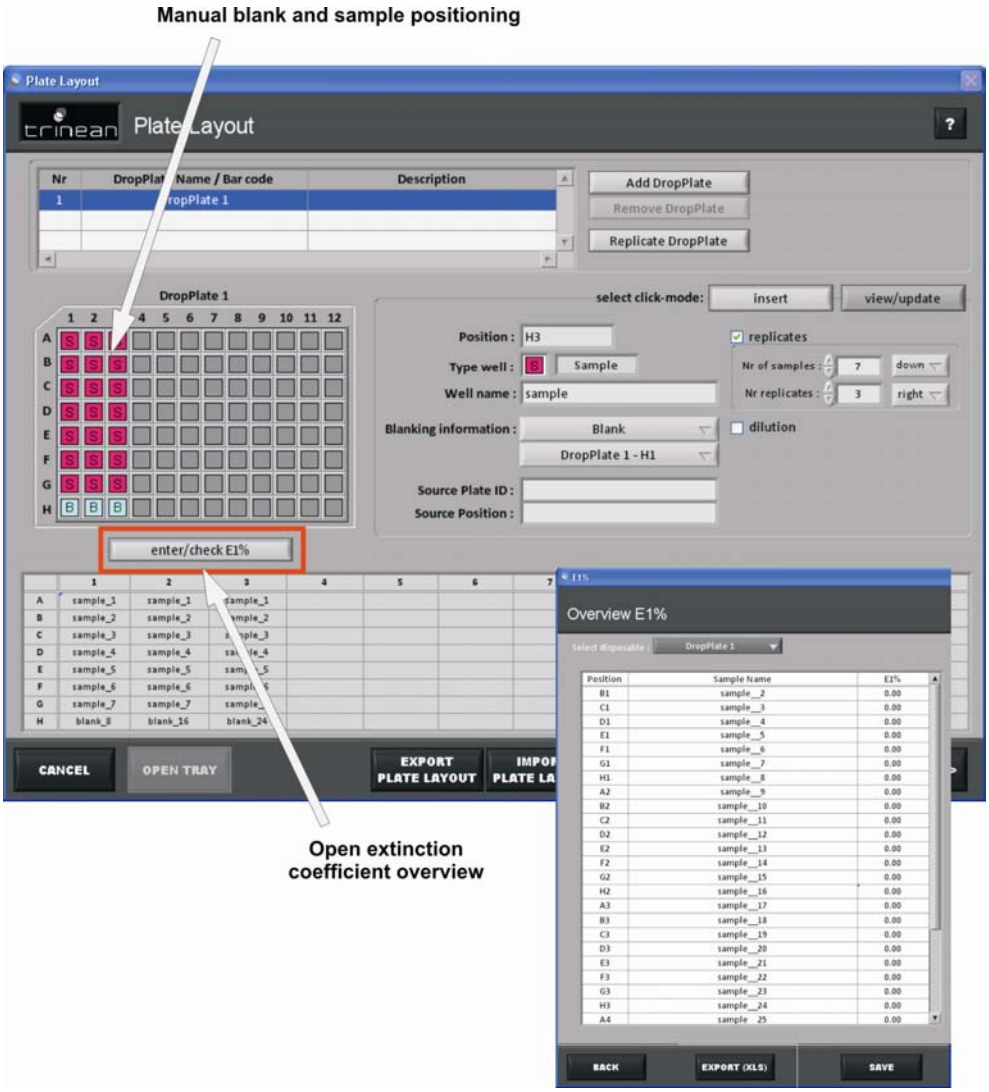


5.2.4 Extra: Measuring multiple proteins with different extinction coefficients

Using the DropSense96 and its DropQuant software, a collection of different proteins (with different UV absorbance properties) can be measured using a single experiment definition.

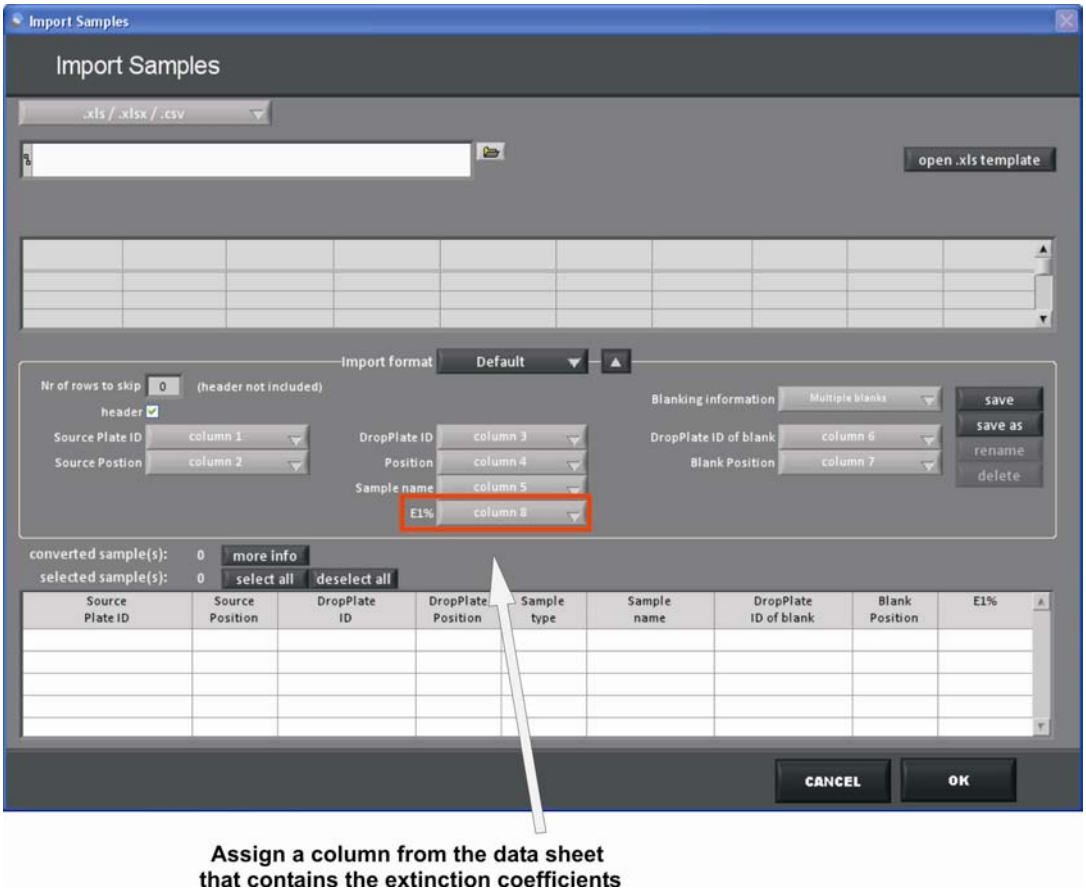
Continue on the 'Start new measurement' screen. Fill in this screen as described in section 4.1.2. Choose as 'Sample type': Purified proteins and as 'Sample material': Multiple proteins. Other options like solvent can be selected as for other proteins (see 4.2.2). No information about extinction coefficients is displayed on the right side as this information is protein dependent. Entering the extinction coefficient of the protein collection can be done manually or be imported.

Continu to the 'Plate layout' screen. When entering the blank and sample positions manually on the plate layout screen, proceed as described in section 5.1.3, and press then press the 'Enter/check E1%' button. Now, a table will appear with a overview of the selected wells. Type in the proper E1% for each protein or copy/past them from another table. All the information can be saved in excel by pressing the 'export excel' button at the bottom of the screen. Press 'Save' to return to the 'Plate layout' screen.



When using the import option (section 4.1.4.2), an extra column selection can be done so the software can import all E1% extinction coefficients for the protein samples. Upon returning to the plate layout screen, an overview of these E1% coefficients can be shown by clicking on the 'enter/check E1%' button.

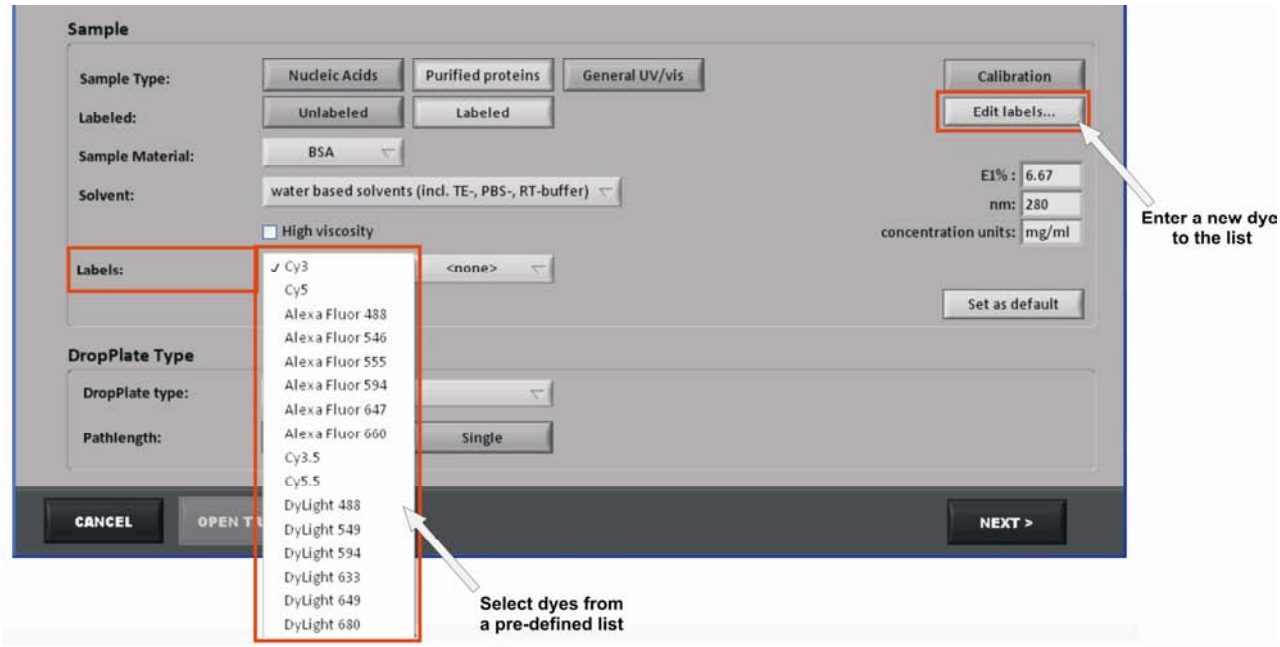
When using the 'Replicates' function (see section 4.1.4.1), entering the extinction coefficient of one protein sample will automatically lead to the fill in of the extinction coefficient of its replicates.



5.2.5 Extra: quantification of fluorescent labeled proteins

The labeling efficiency of fluorescent labeled proteins used in protein arrays can be easily determined with the DropSense96. A full spectrum scan with the DropSense96 allows measurement of protein absorption at 280 nm while detecting the incorporated fluorescent dye at their absorption peak. In a single mesurement, the DropQuant software calculates the protein concentration is expressed in mg/ml and the dye concentrations in pmol/µl.

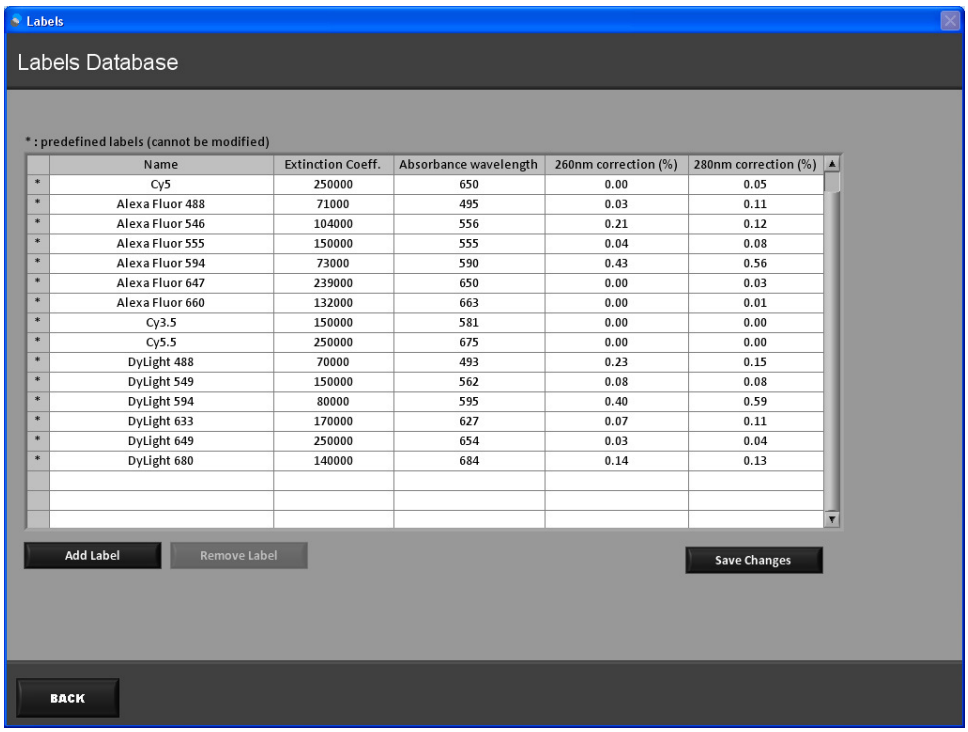
Continue on the 'Start new measurement' screen. Fill in this screen as described in section 4.1.2. Choose as 'sample type': 'Purified proteins' and choose the option 'Labeled'. Other options like solvent can be selected as for other proteins (see 4.2.2).



With selecting the option 'Labeled', two drop down lists appear, each containing a wide range of commercially available fluorescent dyes. Use these drop-down lists to select the appropriate dye (or dyes). If the protein has been labeled with only one dye, choose None as the dye type for the second list.

To enter a new dye, select the 'Edit labels' button on the right side of the screen. This will open a new screen for manual entry of the dye information. Press the 'Add label' button to enter the name and properties given by the manufacturer, like the appropriate correction factors, can be entered. The 280 nm correction will be utilized for protein concentration calculation when the respective dye is selected. To remove a dye, select the dye and press the 'Remove label' button. Pre-defined dyes, identified by a '*' next to the label name may not be edited or deleted.

Press the ‘Save changes’ button and then ‘Back’ to return to the ‘New measurement’ page.

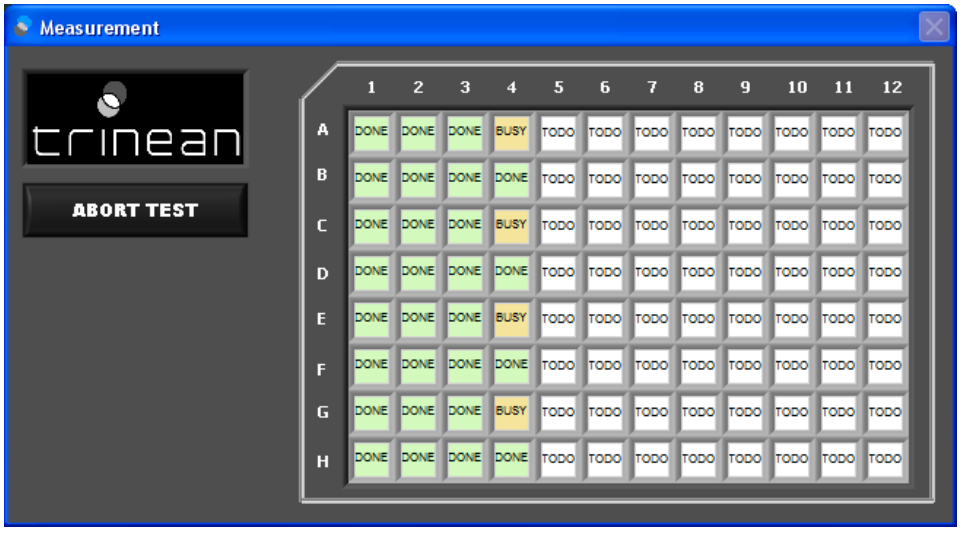


5.2.6 Start measurement

Press the ‘Start Test’ button on the task bar of the ‘Plate layout’ screen. This will open the microplate tray of the connected DropSense96 and the following screen will appear:

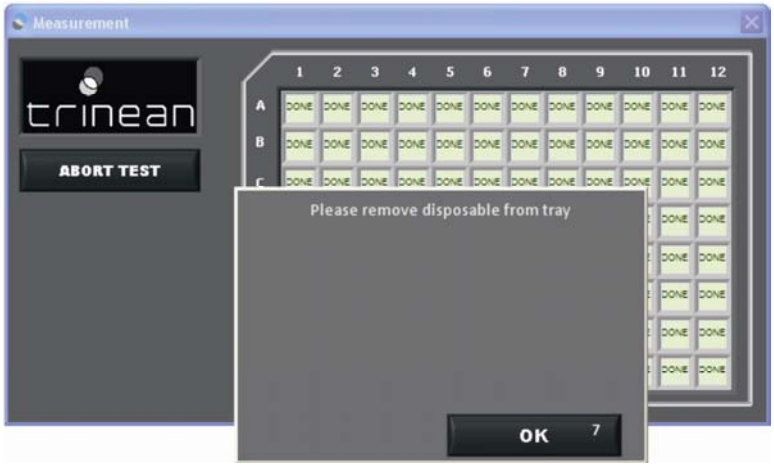
Load the first DropPlate (DropPlate1) on the tray and press ‘OK’. The tray will be pulled in and the measurements will start. Press ‘Cancel’ if the measurement should not take place. The software will return to the main menu.

The progress of a measurement can be observed in the small measurement screen, showing wells that have been measured (indicated by a green color and ‘done’), wells being measured (orange and indicating ‘busy’) and wells that are still waiting to be measured (white color and indicating ‘To do’).

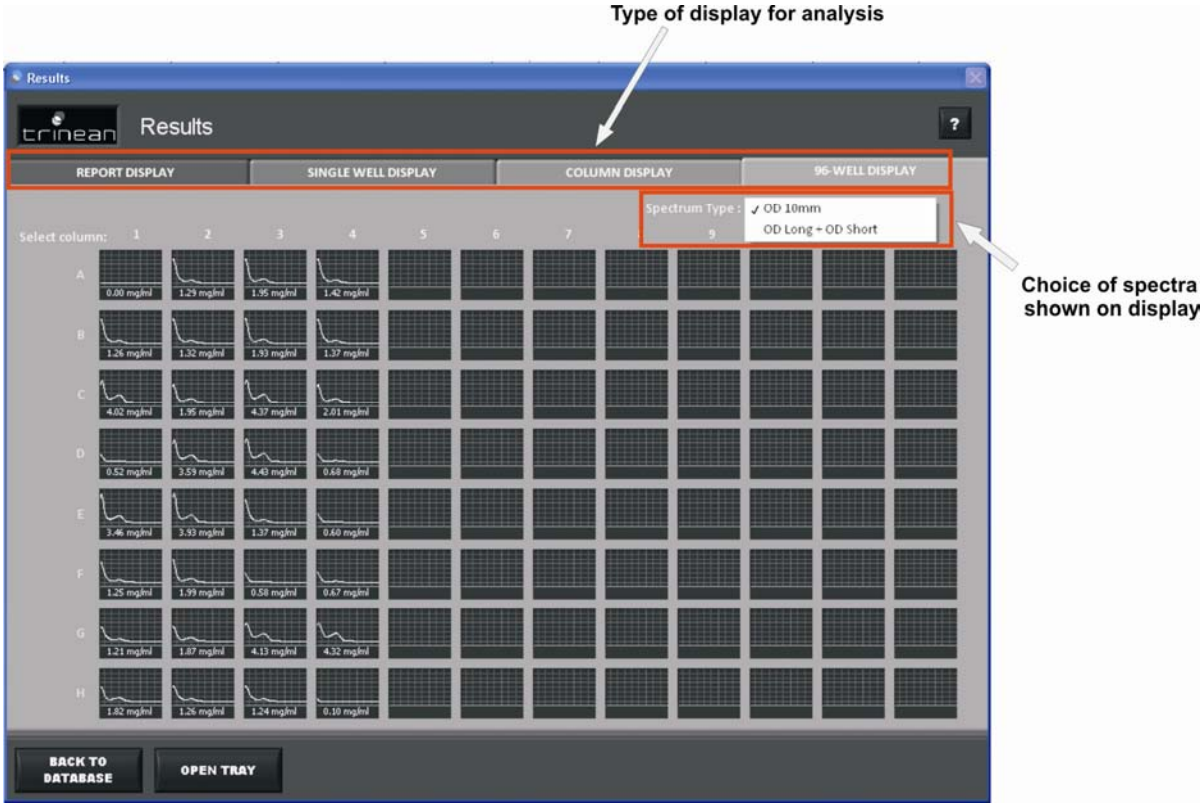

Measurement
trinean
ABORT TEST
1 2 3 4 5 6 7 8 9 10 11 12
A DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
B DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
C DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
D DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
E DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
F DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
G DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
H DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO

5.2.7 Data analysis

When the full measurement is done, following screen appears:

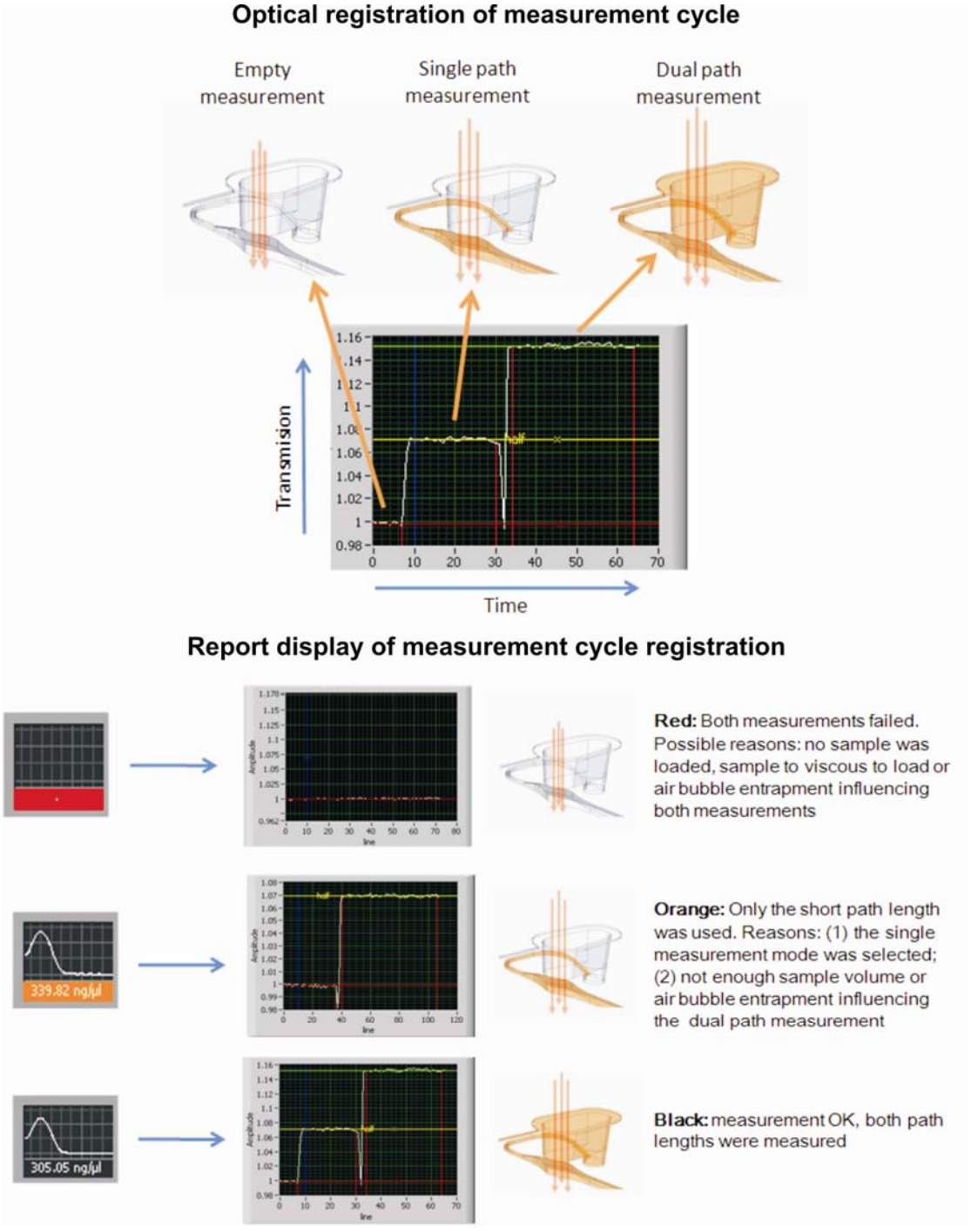


You can take the DropPlate96 of the microplate tray and press 'OK'. Now the software will generate the full spectral graph of all samples and calculate the concentration of the protein samples. For safety reasons, the microplate tray with the DropPlate96 will be pulled in automatically after 30 seconds and the measurements will be displayed. Several display options can be chosen. As default, an 96well overview of the measurements is given.



5.2.7.1 96well display:

Gives an overview of all spectra and the concentration of the nucleic acid calculated from A260 (See example above). In the standard mode, all spectra are shown normalized to a 10mm path. For a DropPlate-D measurement, a combined spectral view of both path lengths can be shown by selecting 'OD long + OD short' in the 'Spectral Type' button. Detailed analysis of one sample can be achieved by clicking on the sample spectrum, opening a detailed figure of this spectrum in the single well display.

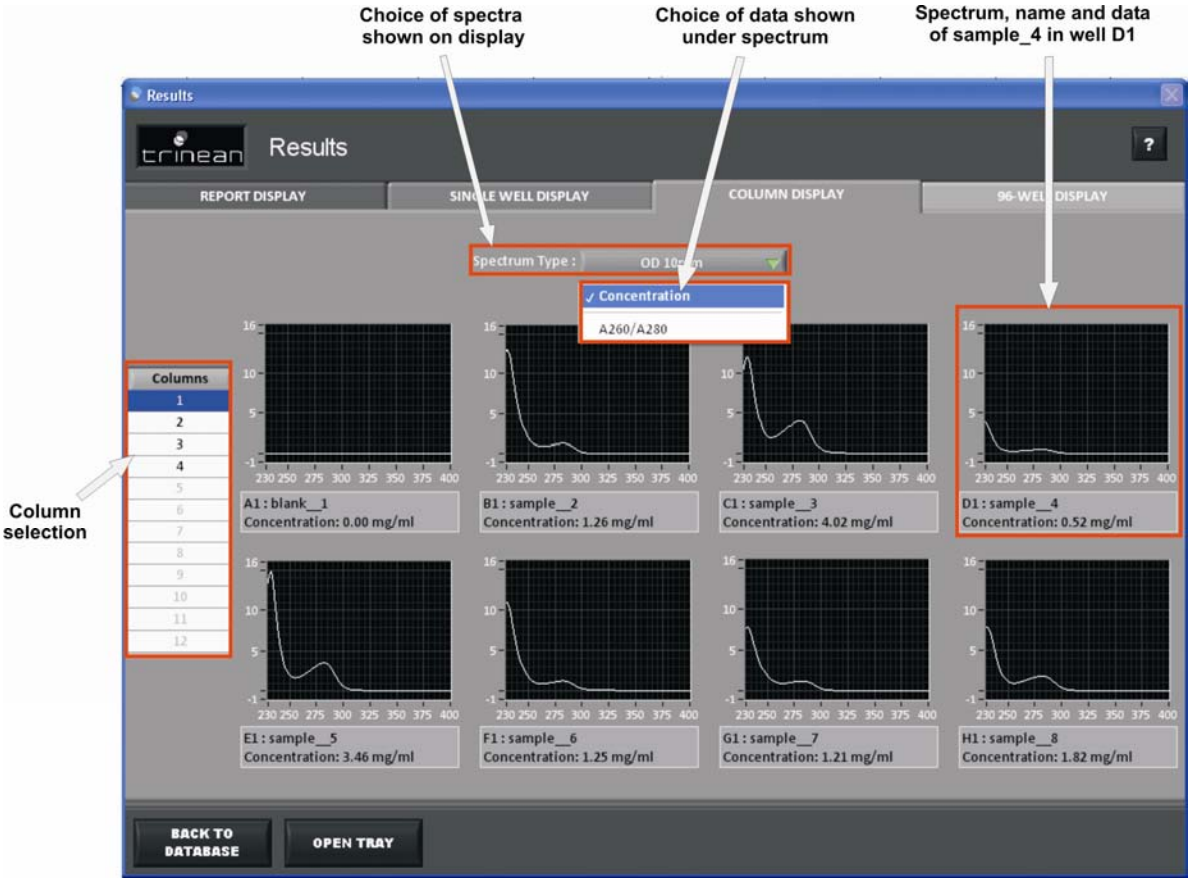

Optical registration of measurement cycle
Empty measurement
Single path measurement
Dual path measurement
Transmision
Time
Report display of measurement cycle registration
339.82 ng/µl
305.05 ng/µl
Red: Both measurements failed. Possible reasons: no sample was loaded, sample to viscous to load or air bubble entrapment influencing both measurements
Orange: Only the short path length was used. Reasons: (1) the single measurement mode was selected; (2) not enough sample volume or air bubble entrapment influencing the dual path measurement
Black: measurement OK, both path lengths were measured

When using **DropPlate-D** consumables, the software will automatically select one of the dual path lenght measurement and normalizes the data to a 1.0 cm (10.0 mm) path. As a general rule: the 1mm pathlenght is used up to OD 1 (OD 10 when normalized t a 1 cm path). Above 1 OD at 1 mm, the concentration is calculated with the 0.2 mm path absorbance measurement.

During the measurement cycle, the transport of the sample through the microfluidic structure is monitored by continuous spectral measuring. This way, errors occuring during the process can be detected and displayed on the screen. This allows a thourough and robust analysis of all measurements. A color legend is used as illustrated on the previous page.

When using **DropPlate-S** consumables, the software will normalize the single cuvette measurement (0.5mm path) to a standard 10.0 mm path. Similar color legends are used as with the DropPlate-D. A black background indicates a good measurement, while a red color inducates a failed measurement. As the DropPlate-S only contains one micro-cuvette, no orange color legend is used.

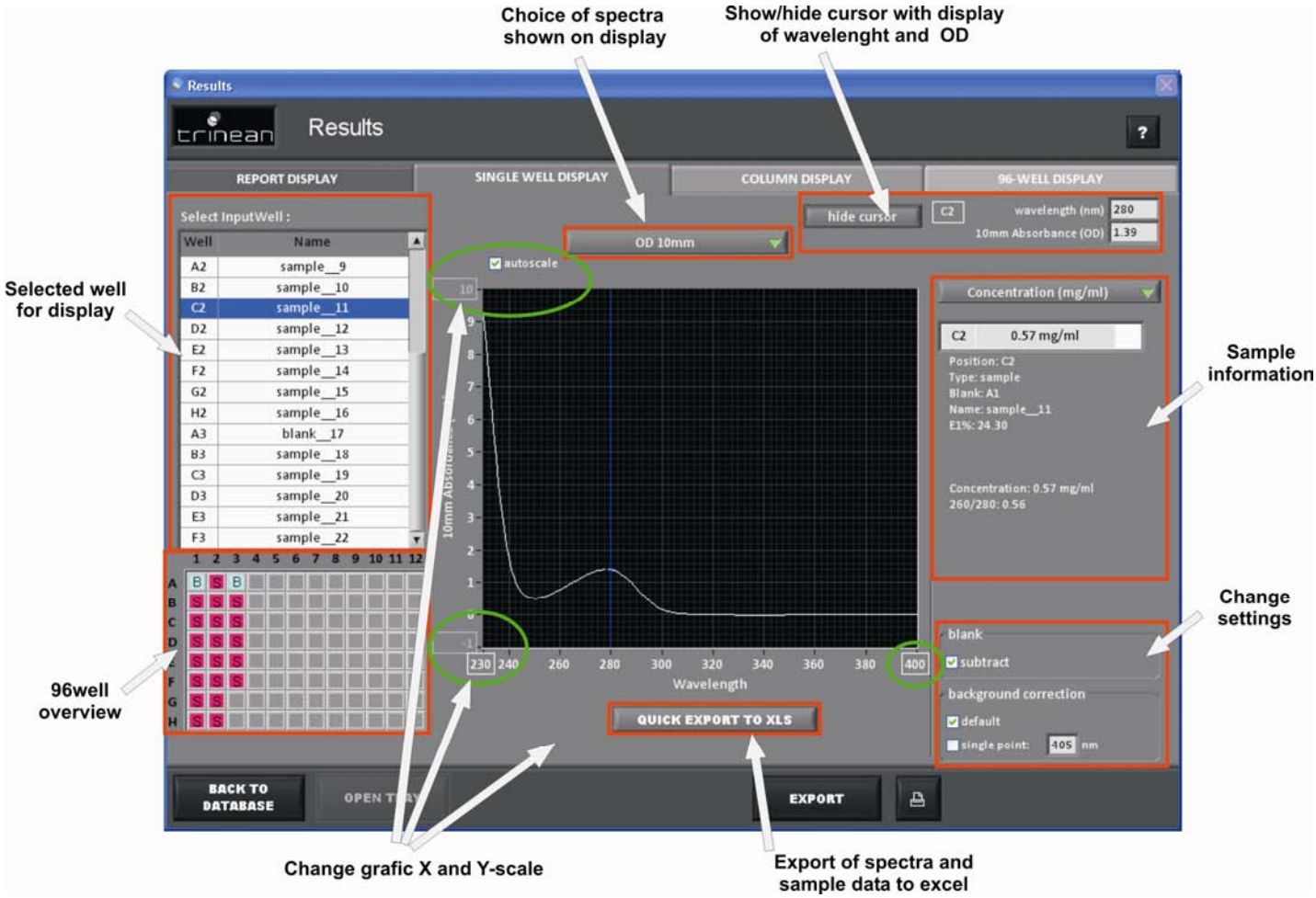
5.2.7.2 Column display:



Gives an overview of all measurements per column. Clicking on another column opens all spectra of this column. Underneath every spectrum, the sample name and data are displayed. A data selection can be done using the scroll button above the spectra. A choice can be made between 'concentration' or 'A260/A280' ratio. The view of the spectra can be changed from 'OD 10mm' to the combined view 'OD long + OD short' using the 'Spectrum Type' button.

5.2.7.3 Single well display

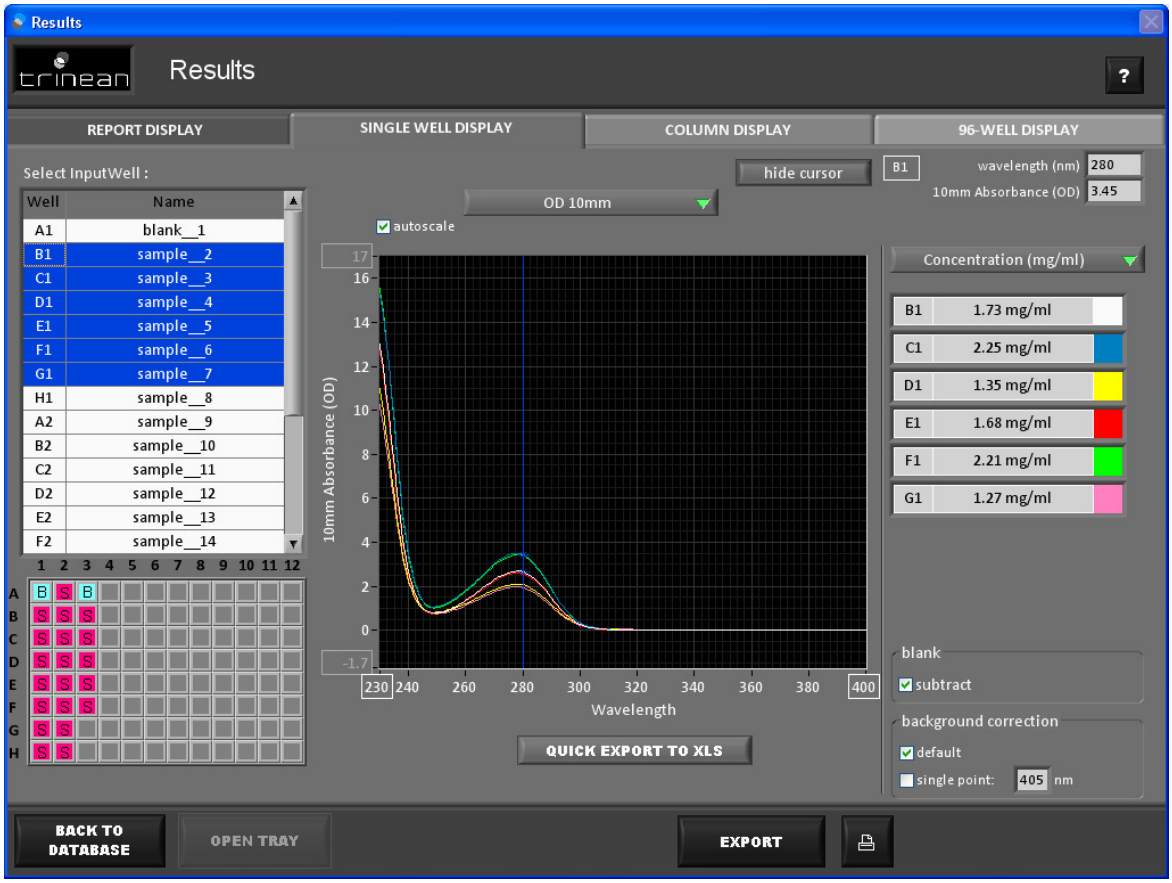
Gives a detailed view of a sample spectrum and all additional sample data next to the spectrum. A sample can be viewed by clicking on the name of the well or sample in the 'select input well' table or by clicking on a well in the 96well overview.



Extra tools are included for detailed analysis and comparison of the samples:

- **Hide/Show cursor**: with this cursor, a specific wavelength can be selected by dragging the blue cursor. The selected wavelength and its OD value (10 mm) is displayed

- **Path length selection**: Above the spectral view, a choice can be made between 'OD 10mm' or 'OD 1.0 mm + OD 0.2mm' (for a DropPlate-D measurement) or 'OD 0.5mm' (for a DropPlate-S measurement). Note that the Y-axis scale will automatically adjust to the proper OD values. When selecting 'OD 1.0 mm + OD 0.2mm', the 1.0mm OD spectra will be shown with a full line, while the 0.2mm OD spectra will be displayed using a dotted line.
- **Overlay spectra:** when pressing the CTRL button on the computer keyboard, select two or more samples from the 'select input well' table to overlay the selected spectra. When using the 'shift' button and selecting two samples, all samples between the selected will also be displayed on the screen.



Additional tools are included to change the analysis settings of the measurements. Changes made within these tools (explianed underneath) stay fixed within the other data analysis screens and the export options.

- **Change scale:** Both the scales of the X-axis and the Y-axis can be altered for a detailed view by changing the highlighted wavelength at the beginning and end of the scale. The setting 'autoscale' has to be turned off when changing Y-axis. Note that the report option 'All absorbance values (10mm)' will show all the absorbance values within the selected wavelength range of the X-axis. So manual changing of the X-axis scale will change the info in the report.
- **Blank subtraction**: When using a blank reference sample, it is automatically subtracted from the sample absorbance before concentration calculation. This feature can be turned off so the spectral shape and concentration calculation is shown without blank compensation. In this mode, the shape of the blank spectrum itself can be analyzed. When the blank subtraction is turned off, all other screens and the report section will generate data without blank subtraction.
- **Background correction**: the background correction method can be selected in the 'start new measurement' sheet (section 5.1.2). The background information is derived from the RAW OD spectrum measured. In the default setting, a linear fit through the 400-600 nm region of the transmission spectrum is subtracted from the full spectrum, while in the single point mode, the background level of a wavelength of choice is subtracted from the full spectrum. The manual selection on the 'single well display' offers the option to chose between the 'default' method, a single wavelength of choice or no background correction. This allows to verify if the background is not near zero as indicator for turbidity or presence of debris. Note that changes in the background correction will be used in the other screens and report section. In the report section, the RAW OD value of the wavelength shown in the single point background correction (for instance RAW A405 (10 mm)) can be included in the report table to export.

The 'single well display' offers a '**quick export to XLS**' function located underneath the spectral view. This function is limited to an export to Excel, showing all the spectra selected on the 'single well display' and a fixed selection of columns with all necessary data from the selected samples.

An example is shown on the next page

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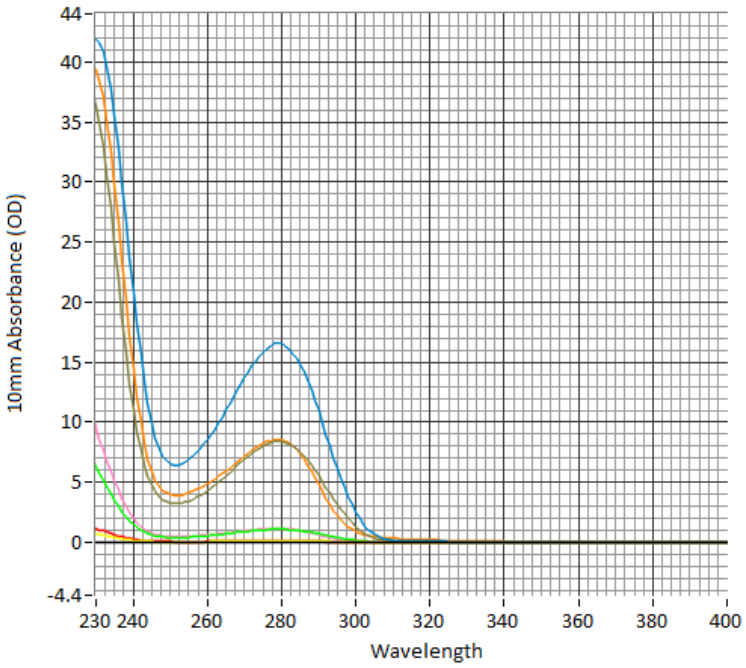
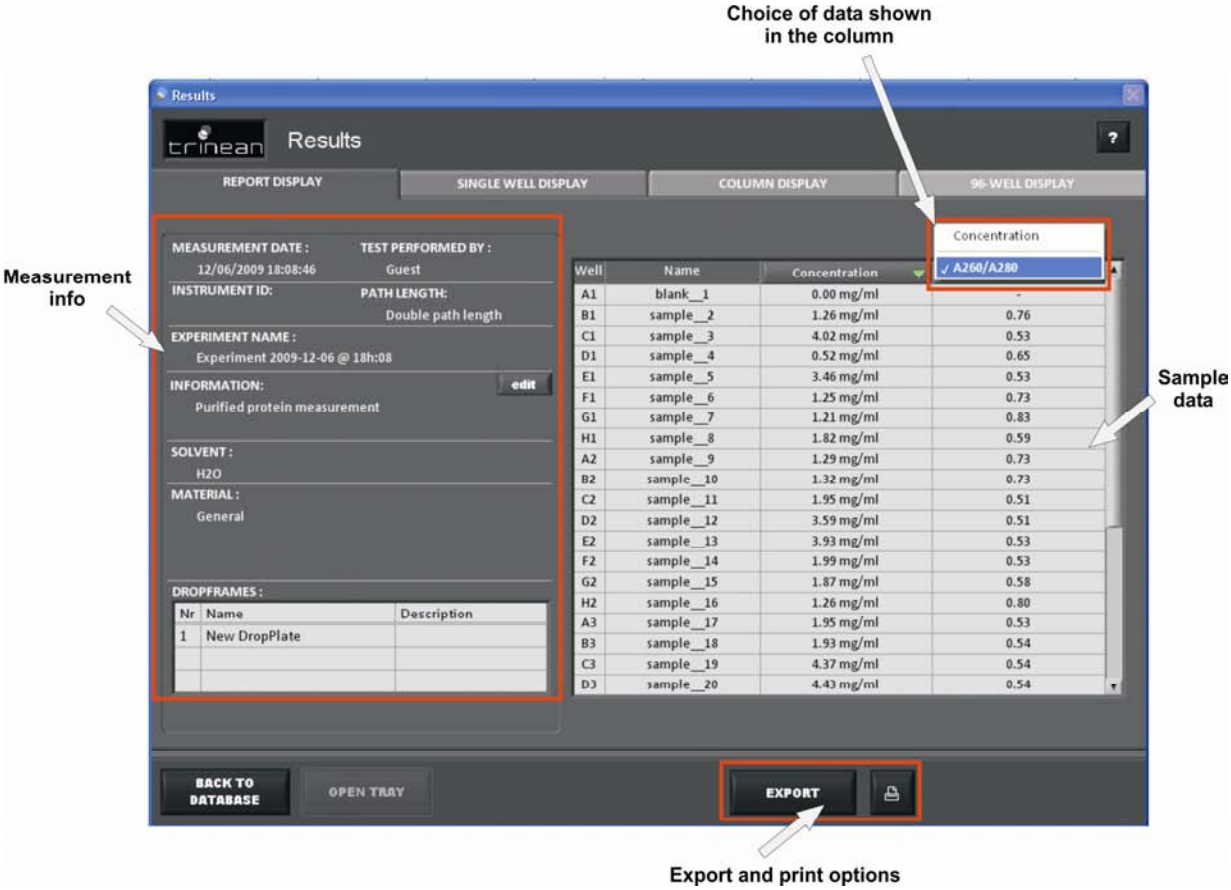
DropSense REPORT



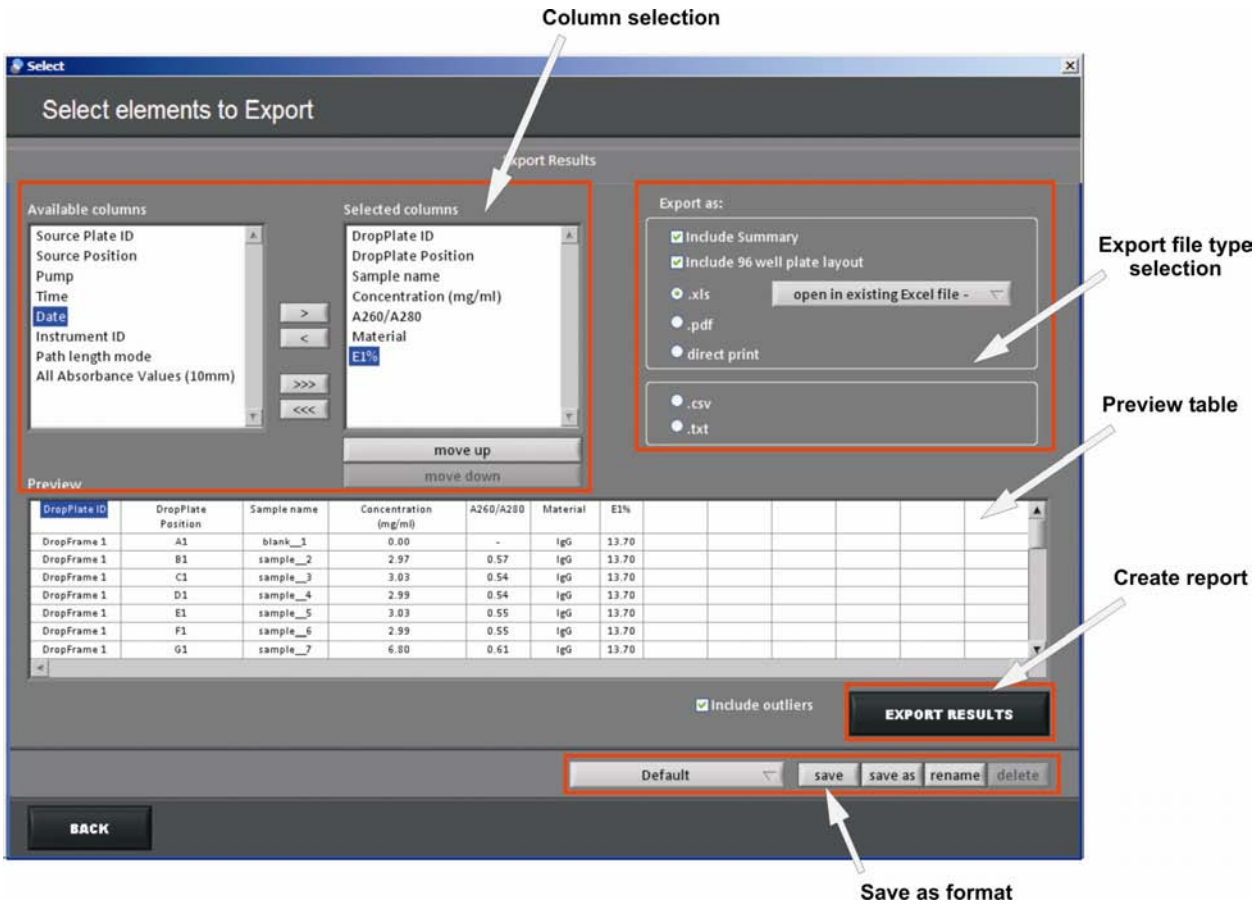
	DropPlate ID	DropPlate Position	Sample name	Pump	Concentration (mg/ml)	A260 (OD 10mm)	A280 (OD 10mm)	Raw A405 (OD 10mm)	A260/A280
	DropPlate 1	A1	PBS		0	0	0	0.01	-2.82
	DropPlate 1	B1	IgG_12.5mg/ml		12.08	16.55	16.55	0.04	0.52
	DropPlate 1	C1	IgG_0.1mg/ml		0.09	0.12	0.12	0.02	0.49
	DropPlate 1	D1	BSA_0.2mg/ml		0.11	0.14	0.14	0.02	0.45
	DropPlate 1	E1	IgG_0.8mg/ml		0.79	1.08	1.08	0.01	0.5
	DropPlate 1	F1	BSA_1.6mg/ml		0.83	1.14	1.14	0.01	0.55
	DropPlate 1	G1	IgG_6.3mg/ml		6.11	8.37	8.37	0.04	0.51
	DropPlate 1	H1	BSA_12.5mg/ml		6.24	8.54	8.54	0.05	0.57

5.2.7.4 Report display

The report section gives an overview of the experiment information and all the measurement data in a table format. The info on the data table can be altered by clicking on the header of the column, concentration, 260/230 or 260/280 ratio's can be chosen. Furthermore, the measurement description can be altered by pressing the 'edit' button. This overview can be exported to several formats like a CSV, txt, excel sheet or pdf file. The data can also be printed directly to a printer of choice.



The 'Export' button opens a new screen to create a data report as desired. In the **results export sheet**, a selection of columns can be made to be included in all three report types. The order of the columns can be altered with the 'move up' and 'move down' buttons. A preview of the report table is displayed at the bottom of the screen. The export file type can be selected by clicking on the preferred file type. Furthermore, the information from the summary (experiment description, experiment name, solvent, …) and an overview of the plate layout can also be included by activating the 'include summary' button. The report design can be saved in a format for future use by clicking the 'save as' button.


Column selection
Select
Select elements to Export
Export Results
Available columns
Source Plate ID
Source Position
Pump
Time
Date
Instrument ID
Path length mode
All Absorbance Values (10mm)
Selected columns
DropPlate ID
DropPlate Position
Sample name
Concentration (mg/ml)
A260/A280
Material
E1%
move up
move down
Export as:
Include Summary
Include 96 well plate layout
.xls
open in existing Excel file -
.pdf
direct print
.csv
.txt
Export file type selection
Preview table
Preview
DropPlate ID | DropPlate Position | Sample name | Concentration (mg/ml) | A260/A280 | Material | E1%
DropFrame 1 | A1 | blank__1 | 0.00 | - | IgG | 13.70
DropFrame 1 | B1 | sample__2 | 2.97 | 0.57 | IgG | 13.70
DropFrame 1 | C1 | sample__3 | 3.03 | 0.54 | IgG | 13.70
DropFrame 1 | D1 | sample__4 | 2.99 | 0.54 | IgG | 13.70
DropFrame 1 | E1 | sample__5 | 3.03 | 0.55 | IgG | 13.70
DropFrame 1 | F1 | sample__6 | 2.99 | 0.55 | IgG | 13.70
DropFrame 1 | G1 | sample__7 | 6.80 | 0.61 | IgG | 13.70
Include outliers
EXPORT RESULTS
Create report
Default
save
save as
rename
delete
BACK
Save as format

5.3 General UV-Vis spectrophotometry

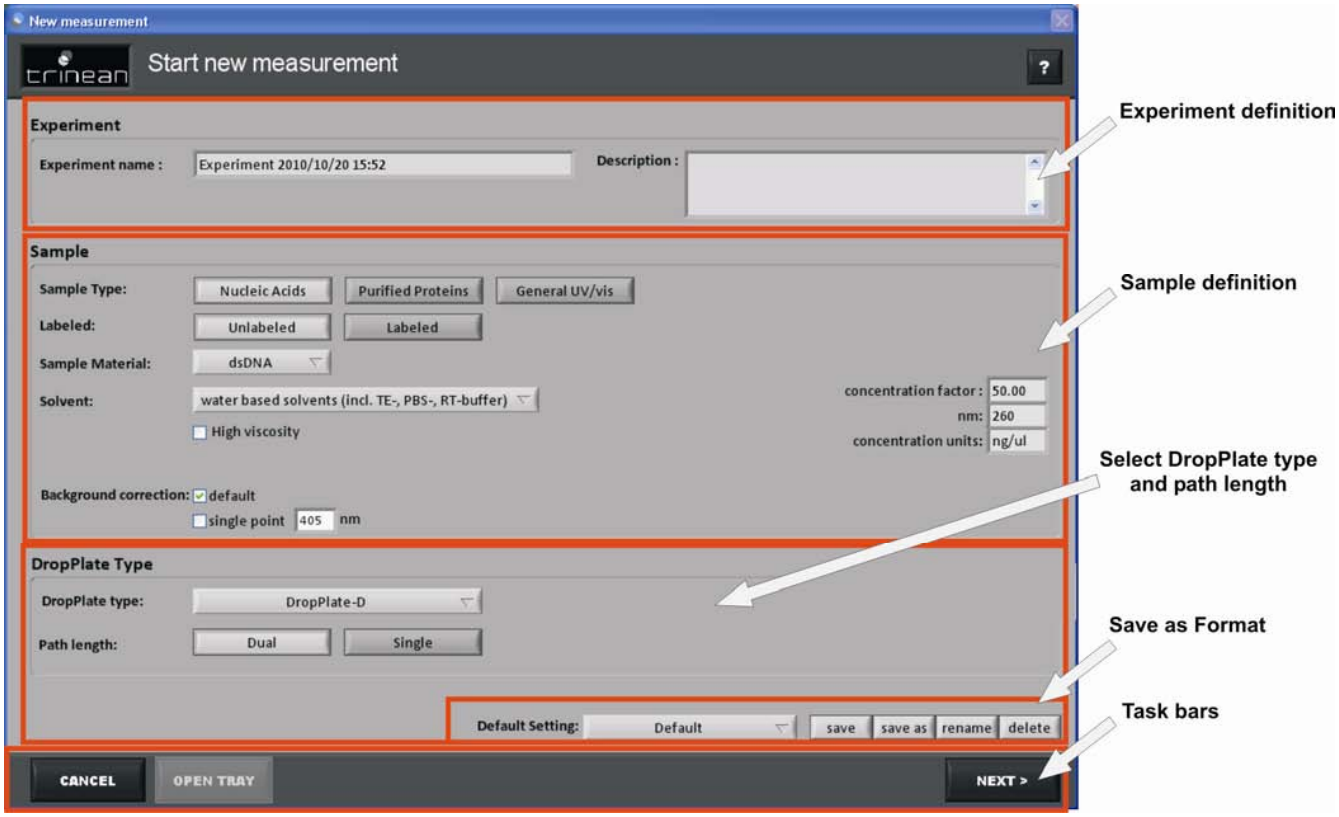
5.3.1 Introduction

In the general UV-Vis application, the DropSense96 functions as a conventional spectrophotometer. An absorbance scan from 230 to 750 nm of any liquid samples can be analyzed, enabling simple identification of absorption peak heights and positions. An unlimited number of wavelenghts can be designated in advance for absorbance monitoring and inclusion in the report.

Note that the values reported are NOT nomalized to a 10mm pathlength, so if comparison is being made to other systems the appropriate factor should be used to convert them (x10 for 1mm pathlength, x50 for 0.2mm path)

5.3.2 Starting a UV-Vis measurement

Pressing the 'new measurement' button on the main menu opens the following screen:



All options on this screen are described in detail in section 4.1.2.

For general UV-Vis measurements, select the proper preferences in the sample definition box:

Sample Type:

Select 'General UV-Vis' (the button turn light gray upon selecting).

Sample material and information:

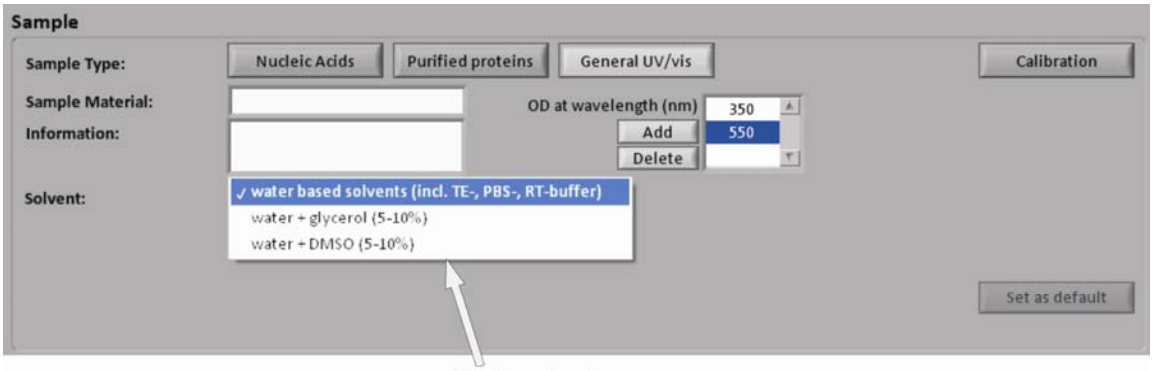
Additional information can be entered, like a name of the solution to be analyzed. An extra text box is foreseen for additional information.

OD at wavelength (nm):

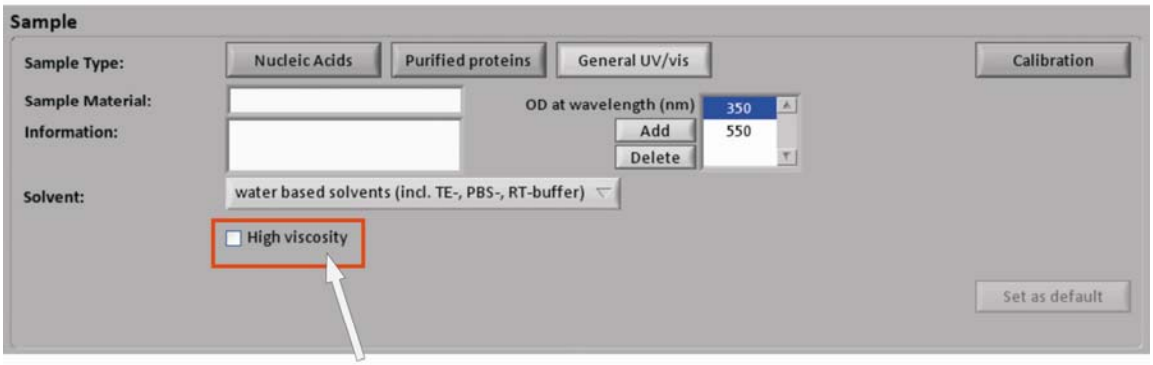
A selection of wavelengths can be designated in the list using the 'Add' and 'Delete' buttons. These wavelengths will be used for OD calculations and this data will be included in the report.

Solvent:

Select the solvent used for the samples to be measured. This solvent information is used by the DropSense96 to fine-tune the pressures used by the vacuum pumps guaranteeing precise and controlled filling of the microfluidic structures. All water-based solvents containing low amounts of salts and buffers are grouped in one option since these substances don't affect the pumping properties.



Choice solvent



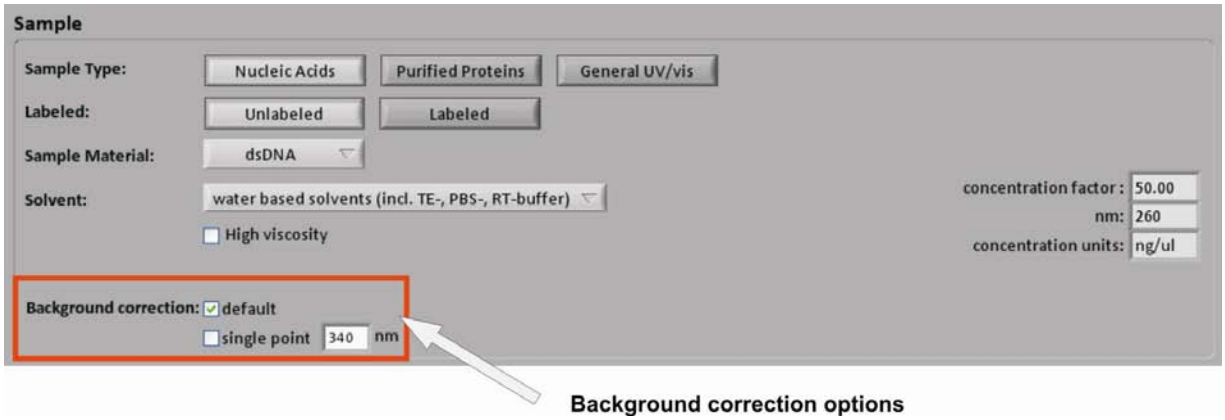
Extra button for high viscous samples

An extra button for high viscous samples can be used, so the pumps will function at higher pressure and longer duration to fill both measuring cuvettes on the DropPlates correctly.

Viscous samples should be handled with care as high viscosity can hamper both precise pipetting of the samples and transport of the sample in the microfluidic DropPlate16.

When the sample is still too viscous, the dual measurement can fail. This will be indicated by an orange or red color indication when the results are shown (see further).
Furthermore, it is prohibited to use other solvents then described in the DropQuant software. Extra caution should be taken with samples containing detergents, as they may have more hydrophobic properties hampering controlled filling of the microfluidic structures.

Background correction:

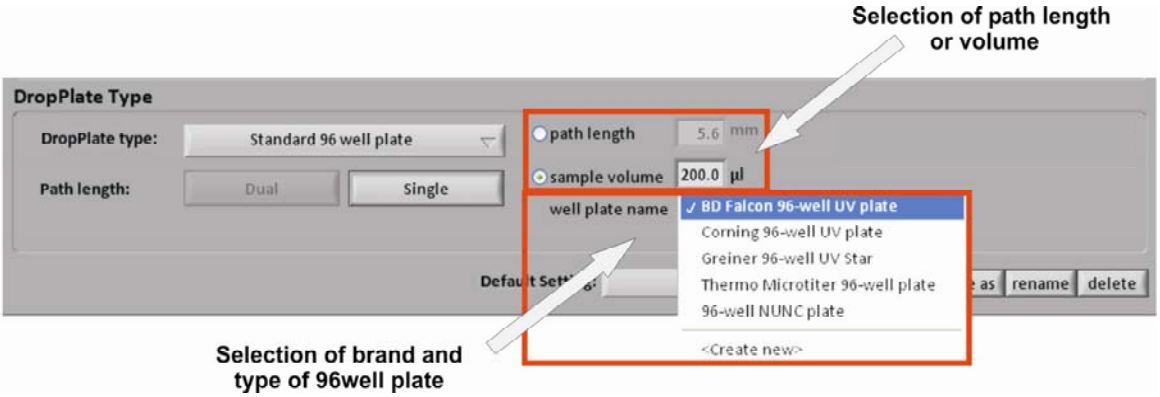


Background correction options

A choice can be made between two types of background corrections. The default background correction determines a linear fit through the 400-600nm part of the spectrum and subtracts this background curve from the full OD range.
The second option is to choose a single wavelength (most common used wavelengths by 'classic' spectrophotometers are 320, 340 and 405 nm). The absorbance value of that wavelength will be subtracted from all wavelength data, so the full spectrum will be set so that the background wavelength of choice will be zero. During the data analysis, the background correction can be altered again or turned off for detailed analysis.

DropPlate selection

Depending on the application or sample concentration range, a choice of DropPlate-D or DropPlate-S can be made. Both DropPlates are explained in detail in section 3.1. Alternatively, a standard 96well microtiter plate can be used. This selection requires additional information like path length or sample volume combined with plate brand and type in order to calculate the correct path length. Additional plate brands and types can be entered by selecting <Create new> from the drop down list.



Path length selection

When using **DropPlate-D** disposables, the user can select between a single or dual path length measurement (see 3.1.2). The single measurement requires 1 µl of sample to perform a single pumping step and a small chamber measurement for high concentration sample analysis (2-110 OD, 10mm equivalent absorbance). The dual measurement, requiring 3 µl of sample, uses both measurement chambers for the analysis creating the large concentration measurement range (0.05-110 OD, 10mm equivalent absorbance).

When using a **DropPlate-S**, only a single measurement is possible. This consumable requires 2 µl of sample and performs a single measurement with a measurement range of 0.05-44 OD, 10mm equivalent absorbance.

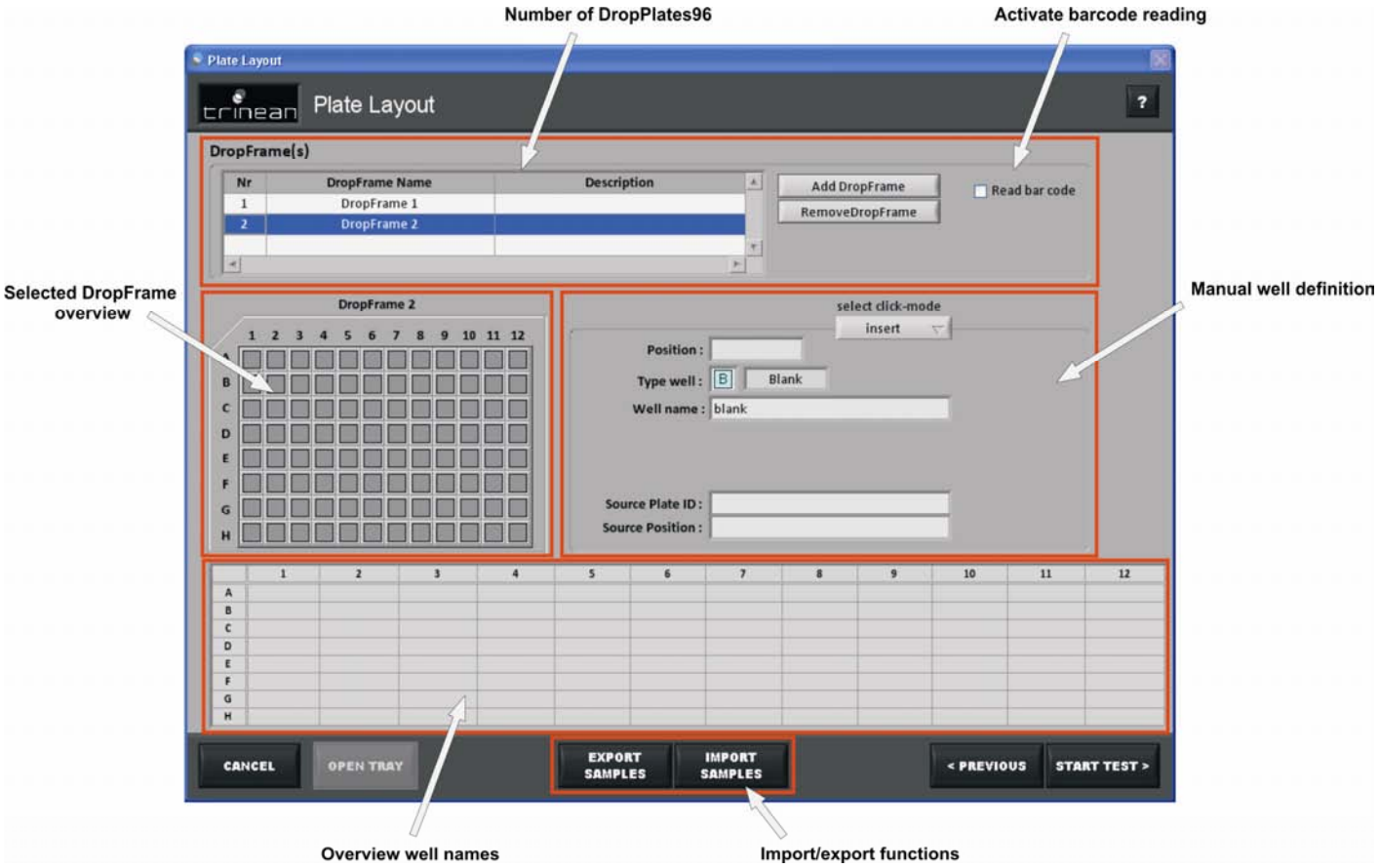
Save as a format:

When all the above selections have been made, the user can save these in a new format for future use. These formats are account dependent. Only the lab manager account has the possibility to make a format that is available for all users. The formats can be deleted, renamed, …

When all sample properties are entered, press the 'next' button on the tast bar below

5.3.3 DropPlate96 sample layout

Pressing the 'next' button on the 'Start new measurement' screen will open the next screen where information on sample data and DropPlates96 layout can be entered. The positioning information of samples or blank can be entered manually or can be imported from an excel, txt or .CSV-file. Both import methods are described in detail in section 4.1.4.



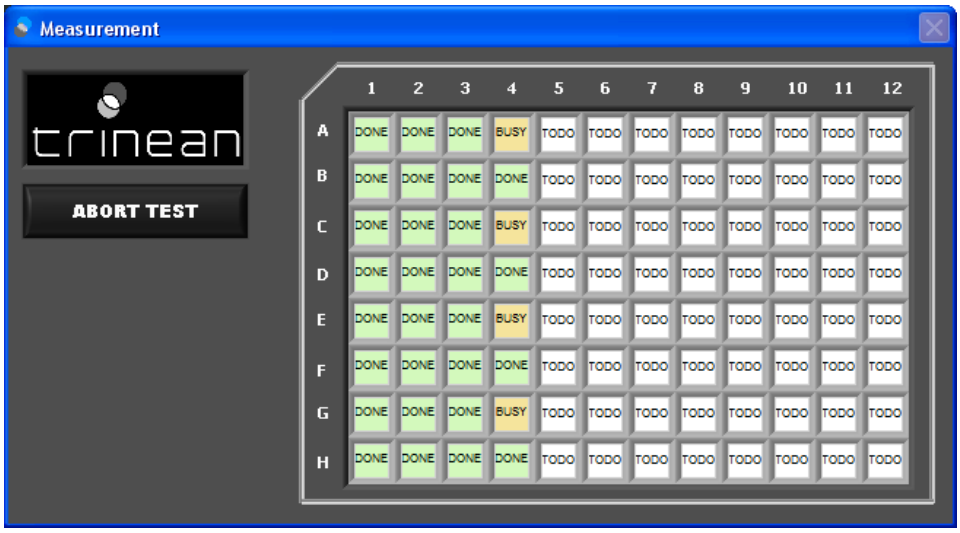
5.3.4 Start measurement

Press the ‘Start Test’ button on the task bar of the ‘Plate layout’ screen. This will open the microplate tray of the connected DropSense96 and the following screen will appear:

Load the first DropPlate (DropPlate1) on the tray and press ‘OK’. The tray will be pulled in and the measurements will start. Press ‘Cancel’ if the measurement should not take place. The software will return to the main menu.

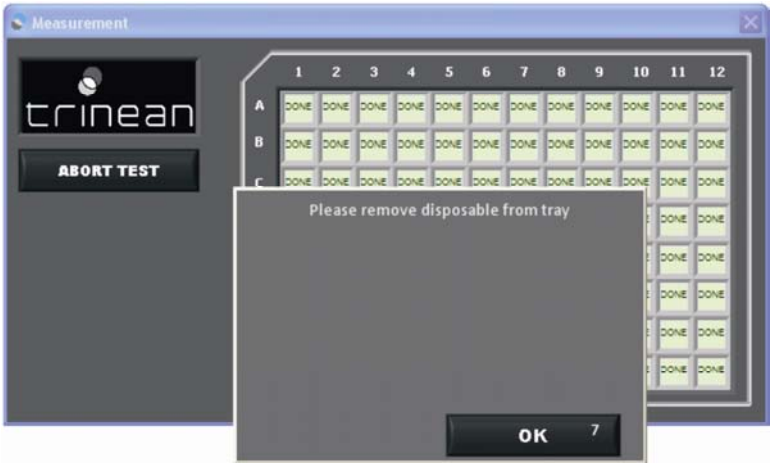
The progress of a measurement can be observed in the small measurement screen, showing wells that are measured (indicated by a green color and ‘done’), wells being measured (orange and indicating ‘busy’) and wells that are still waiting to be measured (white color and indicating ‘To do’).

5.3.5

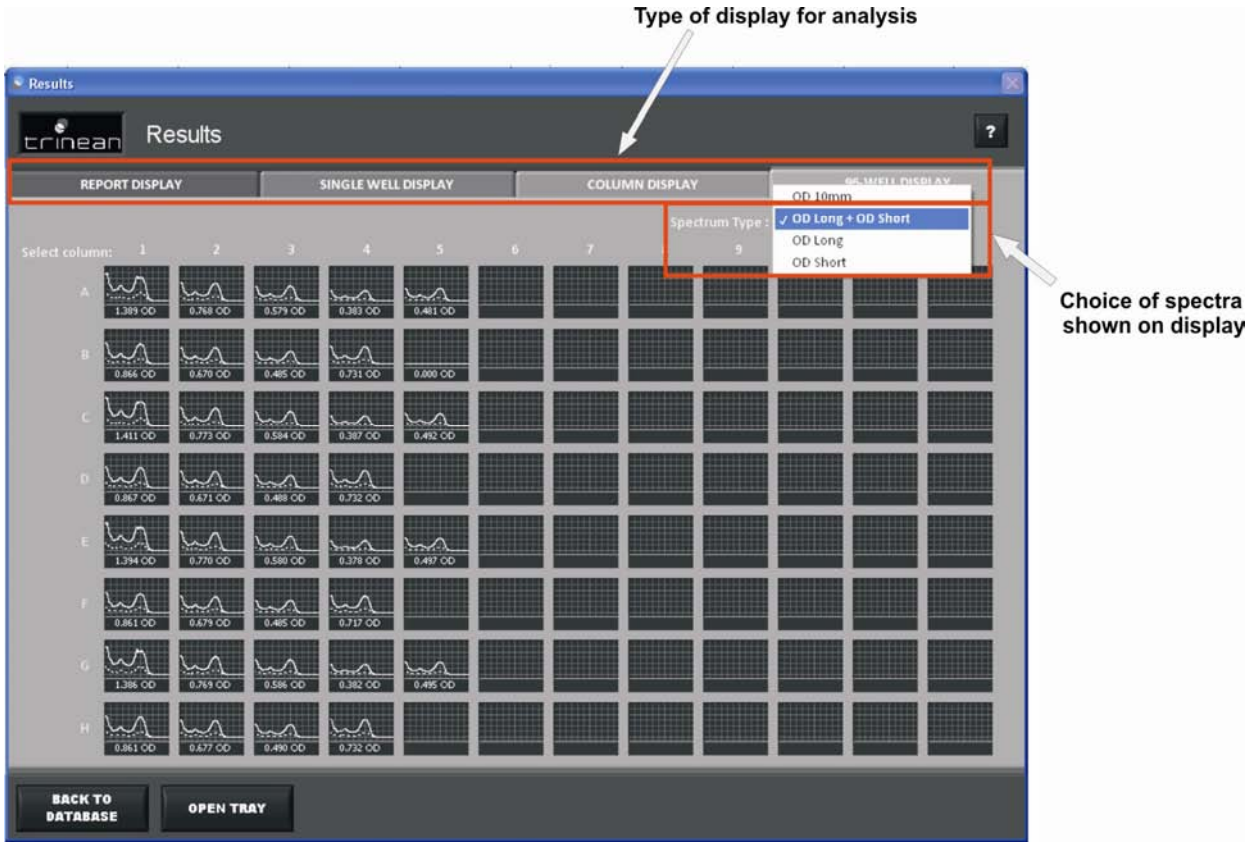

Measurement
trinean
ABORT TEST
1 2 3 4 5 6 7 8 9 10 11 12
A DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
B DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
C DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
D DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
E DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
F DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO
G DONE DONE DONE BUSY TODO TODO TODO TODO TODO TODO TODO TODO
H DONE DONE DONE DONE TODO TODO TODO TODO TODO TODO TODO TODO

Data analysis

When the full measurement is done, following screen appears:

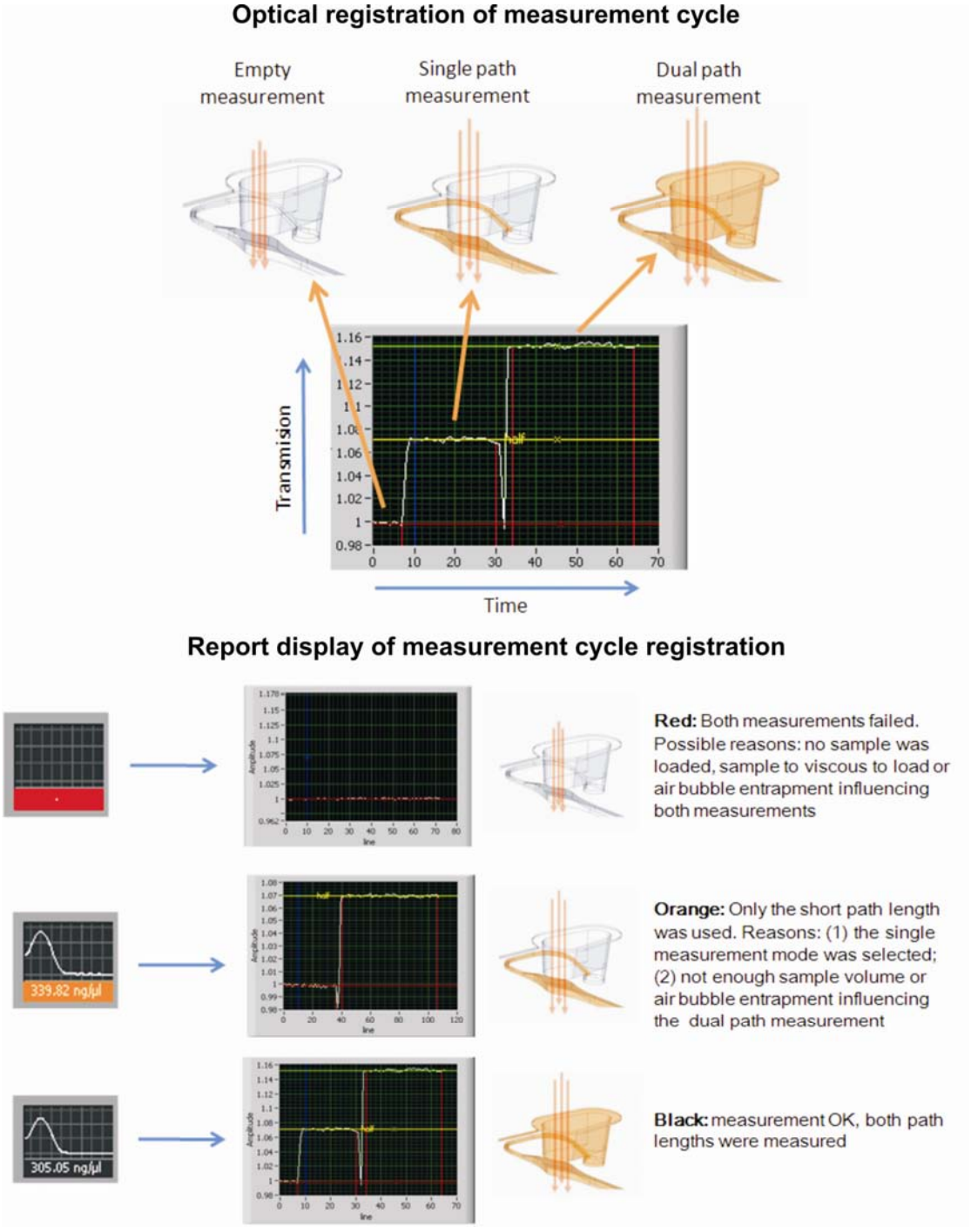


You can take the DropPlate96 of the microplate tray and press 'OK'. Now the software will generate the full spectral scan of all samples and calculate the concentration of the protein samples. For safety reasons, the microplate tray with the DropPlate96 will be pulled in automatically after 30 seconds and the measurements will be displayed. Several display options can be chosen. As default, an 96well overview of the measurements is given.



5.3.5.1 96well display:

Gives an overview of all spectra and the concentration of the nucleic acid calculated from A260 (See example above). In the standard mode, all spectra are shown normalized to a 10mm path. For a DropPlate-D measurement, a combined spectral view of both path lengths can be shown by selecting 'OD long + OD short' in the 'Spectral Type' button. Detailed analysis of one sample can be achieved by clicking on the sample spectrum, opening a detailed figure of this spectrum in the single well display.

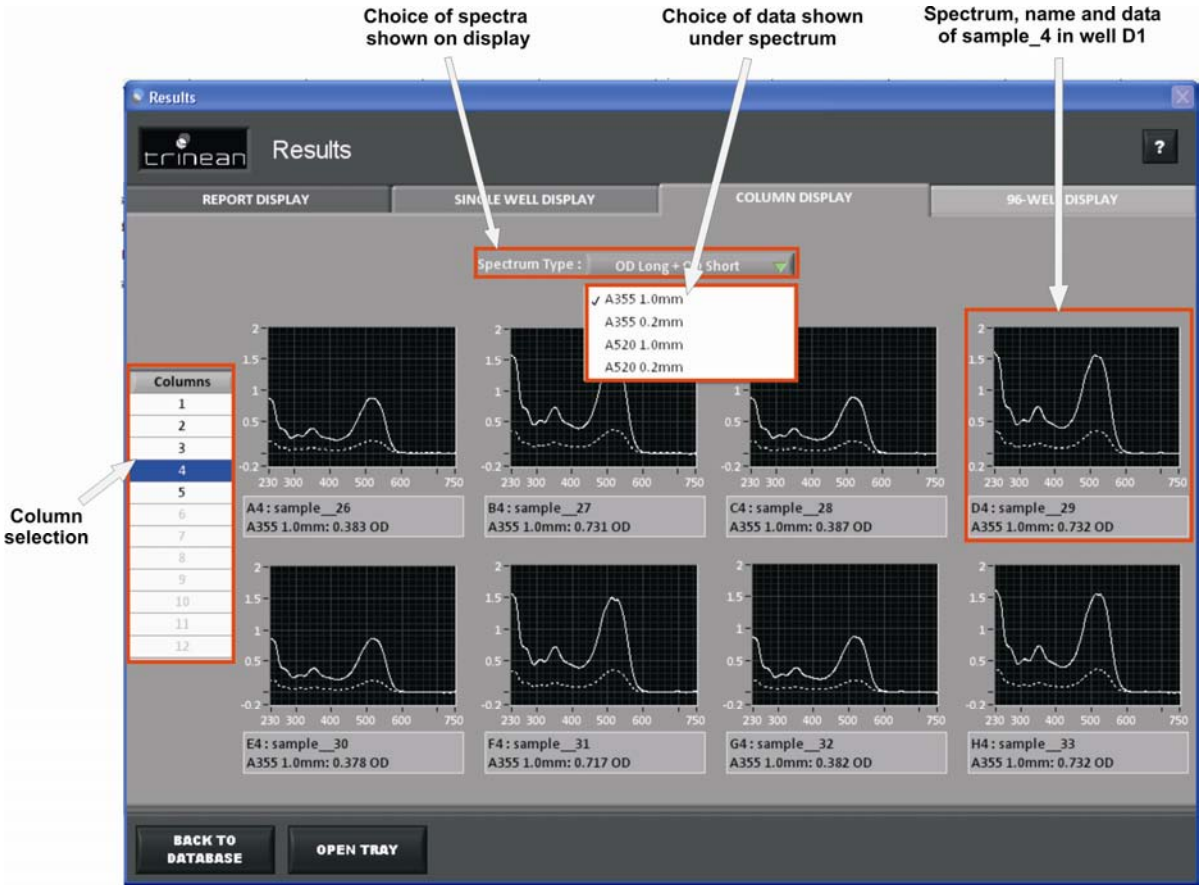

Optical registration of measurement cycle
Empty measurement
Single path measurement
Dual path measurement
Transmision
Time
Report display of measurement cycle registration
339.82 ng/µl
305.05 ng/µl
Red: Both measurements failed. Possible reasons: no sample was loaded, sample to viscous to load or air bubble entrapment influencing both measurements
Orange: Only the short path length was used. Reasons: (1) the single measurement mode was selected; (2) not enough sample volume or air bubble entrapment influencing the dual path measurement
Black: measurement OK, both path lengths were measured

When using **DropPlate-D** consumables, the software will automatically select one of the dual path lenght measurement and normalizes the data to a 1.0 cm (10.0 mm) path. As a general rule: the 1mm pathlenght is used up to OD 1 (OD 10 when normalized t a 1 cm path). Above 1 OD at 1 mm, the concentration is calculated with the 0.2 mm path absorbance measurement.

During the measurement cycle, the transport of the sample through the microfluidic structure is monitored by continuous spectral measuring. This way, errors occuring during the process can be detected and displayed on the screen. This allows a thourough and robust analysis of all measurements. A color legend is used as illustrated on the previous page.

When using **DropPlate-S** consumables, the software will normalize the single cuvette measurement (0.5mm path) to a standard 10.0 mm path. Similar color legends are used as with the DropPlate-D. A black background indicates a good measurement, while a red color inducates a failed measurement. As the DropPlate-S only contains one micro-cuvette, no orange color legend is used

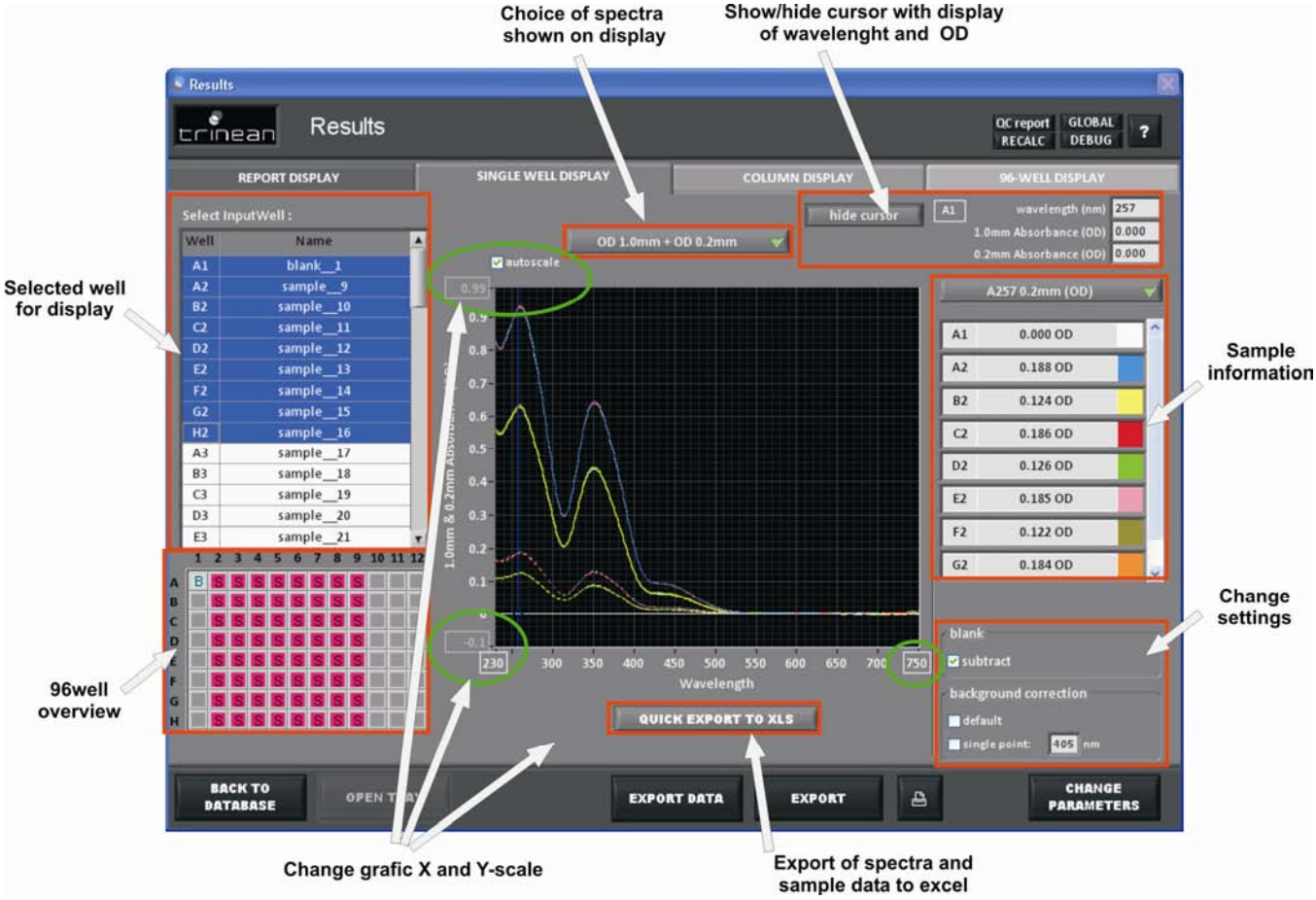
5.3.5.2 Column display:



Gives an overview of all measurements per column. Clicking on another column opens all spectra of this column. Underneath every spectrum, the sample name and data are displayed. A data selection can be done using the drop down box above the spectra. A choice can be made between the OD values of the designated wavelengths. The view of the spectra can be changed using the 'Spectrum Type' button

5.3.5.3 Single well display

Gives a detailed view of a sample spectrum and all additional sample data next to the spectrum. A sample can be viewed by clicking on the name of the well or sample in the 'select input well' table or by clicking on a well in the 96well overview.



Extra tools are included for detailed analysis and comparison of the samples:

- **Hide/Show cursor**: with this cursor, a specific wavelength can be selected by dragging the blue cursor. The selected wavelength and its OD value (10 mm) is displayed
- **Path length selection**: Above the spectral view, a choice can be made between 'OD 10mm' or 'OD 1.0 mm + OD 0.2mm' (default for a DropPlate-D measurement) or 'OD

0.5mm' (default for a DropPlate-S measurement). Note that the Y-axis scale will automatically adjust to the proper OD values. When selecting 'OD 1.0 mm + OD 0.2mm', the 1.0mm OD spectra will be shown with a full line, while the 0.2mm OD spectra will be displayed using a dotted line.

- **Overlay spectra:** when pressing the CTRL button on the computer keyboard, select two or more samples from the 'select input well' table to overlay the selected spectra. When using the 'shift' button and selecting two samples, all samples between the selected will also be displayed on the screen..

Additional tools are included to change the analysis settings of the measurements. Changes made within these tools (explianed underneath) stay fixed within the other data analysis screens and the export options.

- **Change scale:** Both the scales of the X-axis and the Y-axis can be altered for a detailed view by changing the highlighted wavelength at the beginning and end of the scale. The setting 'autoscale' has to be turned off when changing Y-axis. Note that the report option 'All absorbance values (10mm)' will show all the absorbance values within the selected wavelength range of the X-axis. So manual changing of the X-axis scale will change the info in the report.
- **Blank subtraction**: When using a blank reference sample, it is automatically subtracted from the sample absorbance before concentration calculation. This feature can be turned off so the spectral shape and concentration calculation is shown without blank compensation. In this mode, the shape of the blank spectrum itself can be analyzed. When the blank subtraction is turned off, all other screens and the report section will generate data without blank subtraction.
- **Background correction**: the background correction method can be selected in the 'start new measurement' sheet (section 5.1.2). The background information is derived from the RAW OD spectrum measured. In the default setting, a linear fit through the 400-600 nm region of the transmission spectrum is subtracted from the full spectrum, while in the single point mode, the background level of a wavelength of choice is subtracted from the full spectrum. The manual selection on the 'single well display' offers the option to chose between the 'default' method, a single wavelength of choice or no background correction. This allows to verify if the background is not near zero as indicator for turbidity or presence of debris. Note that changes in the background

correction will be used in the other screens and report section. In the report section, the RAW OD value of the wavelength shown in the single point background correction (for instance RAW A405 (10 mm)) can be included in the report table to export.

The 'single well display' offers a '**quick export to XLS**' function located underneath the spectral view. This function is limited to an export to Excel, showing all the spectra selected on the 'single well display' and a fixed selection of columns with all necessary data from the selected samples.

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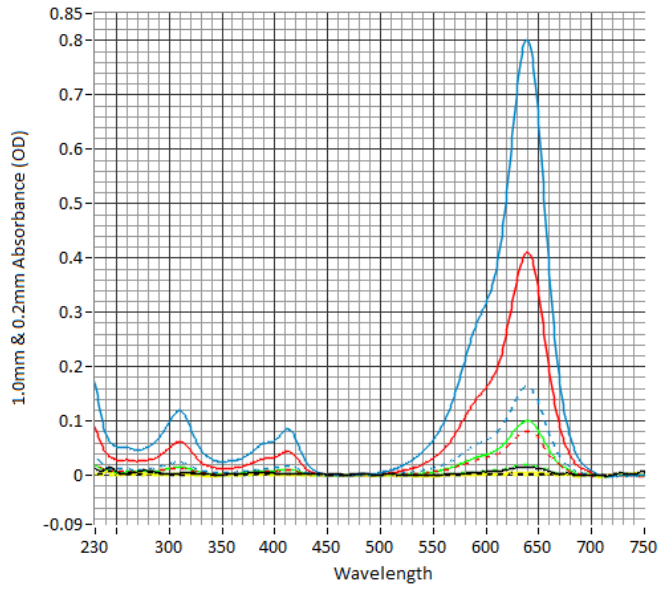
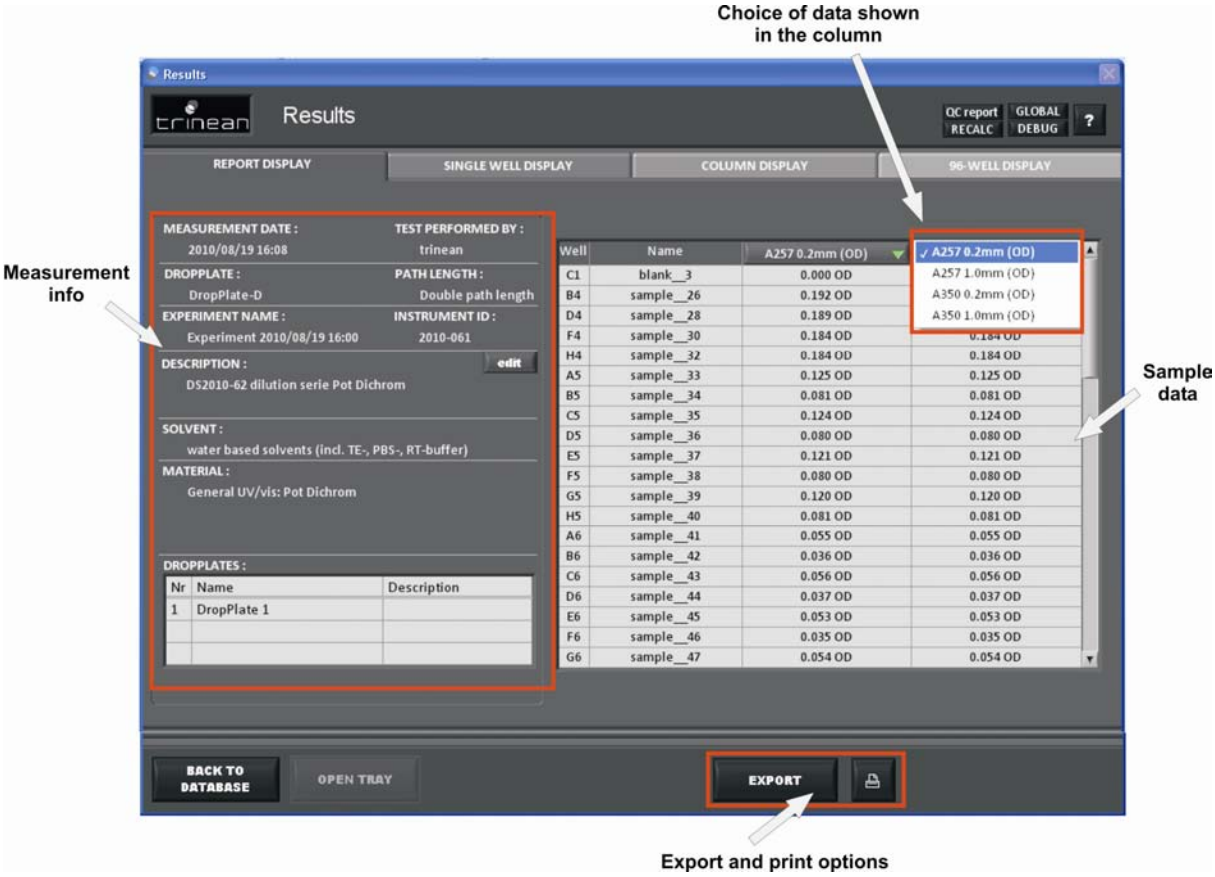
DropSense REPORT



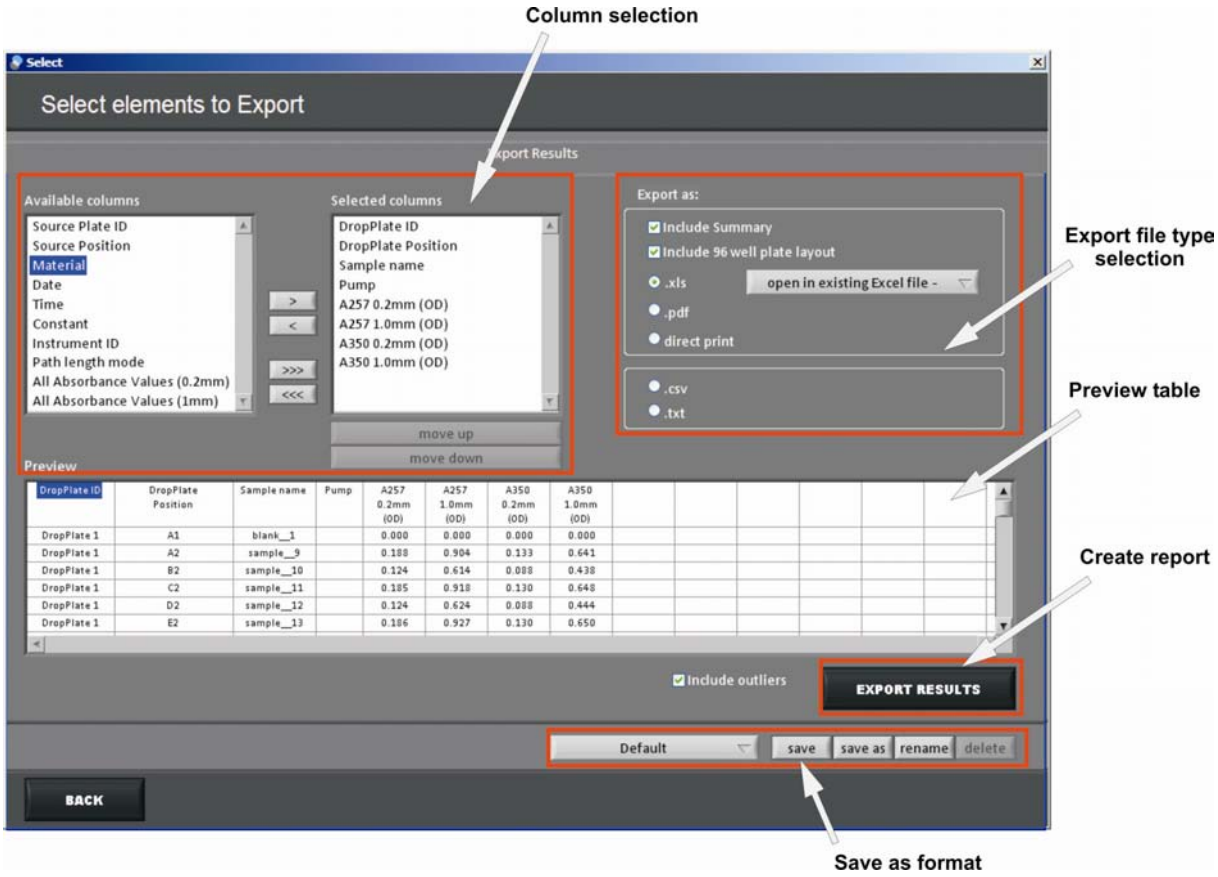
	DropPlate ID	DropPlate Position	Sample name	Pump	A638 (OD 10mm)	A638 (OD 0.2mm)	A638 (OD 1mm)	A411 (OD 10mm)	A411 (OD 0.2mm)	A411 (OD 1mm)	Raw A405 (10.000000)	Raw A405 (0.200000)	Raw A405 (1.000000)
	DropPlate 1	E5	2		0.14	0.004	0.014	0.034	0.002	0.003	0.048	0.003	0.005
	DropPlate 1	F5	8		8.125	0.164	0.801	0.849	0.018	0.085	0.811	0.017	0.081
	DropPlate 1	G5	1		0.052	0.001	0.005	0.005	0.001	0.001	0.005	0	0
	DropPlate 1	H5	7		4.097	0.081	0.41	0.437	0.01	0.044	0.422	0.01	0.042
	DropPlate 1	A6	5		1.001	0.02	0.1	0.108	0.003	0.011	0.098	0.002	0.01

5.3.5.4 Report display

The report section gives an overview of the experiment information and all the measurement data in a table format. The info on the data table can be altered by clicking on the header of the column. Furthermore, the measurement description can be altered by pressing the 'edit' button. This overview can be exported to several formats like a CSV, txt, excel sheet or pdf file. The data can also be send directly to a printer of choice.



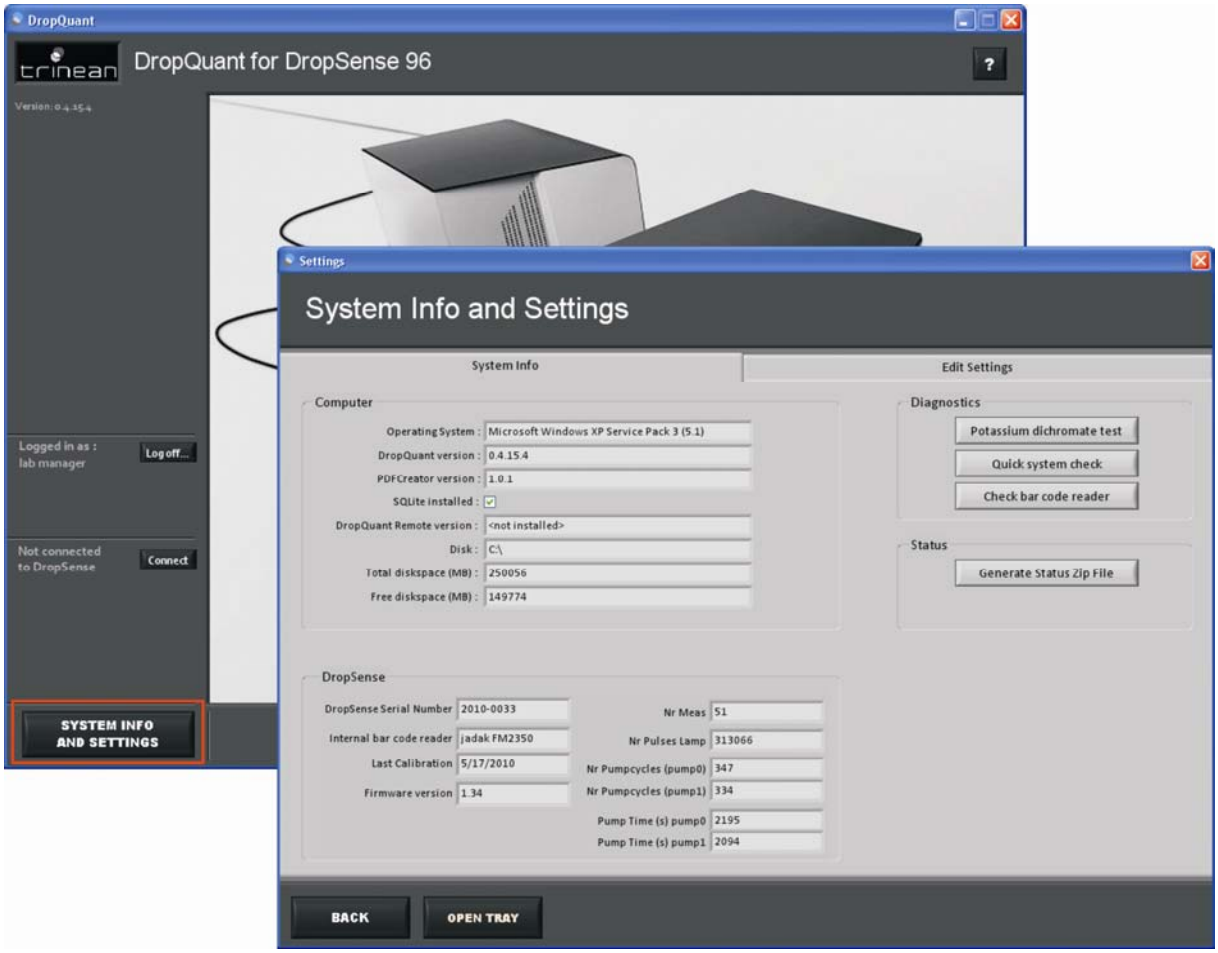
The 'Export' button opens a new screen to create a data report as desired. In the **results export sheet**, a selection of columns can be made to be included in all three report types. The order of the columns can be altered with the 'move up' and 'move down' buttons. A preview of the report table is displayed at the bottom of the screen. The export file type can be selected by clicking on the preferred file type. Furthermore, the information from the summary (experiment description, experiment name, solvent, …) and an overview of the plate layout can also be included by activating the 'include summary' button. The report design can be saved in a format for future use by clicking the 'save as' button.


Column selection
Select
Select elements to Export
Available columns
Source Plate ID
Source Position
Material
Date
Time
Constant
Instrument ID
Path length mode
All Absorbance Values (0.2mm)
All Absorbance Values (1mm)
Selected columns
DropPlate ID
DropPlate Position
Sample name
Pump
A257 0.2mm (OD)
A257 1.0mm (OD)
A350 0.2mm (OD)
A350 1.0mm (OD)
move up
move down
Export as:
Include Summary
Include 96 well plate layout
.xls
open in existing Excel file -
.pdf
direct print
.csv
.txt
Export file type selection
Preview table
Preview
Create report
Include outliers
EXPORT RESULTS
Default
save
save as
rename
delete
BACK
Save as format

6. Diagnostics

6.1 System Info and Settings

The diagnostic tools are only available for a log-in with an entry level as 'lab manager'. A extra software button is located on the left bottom side main screen: 'Systems Info and Settings'.



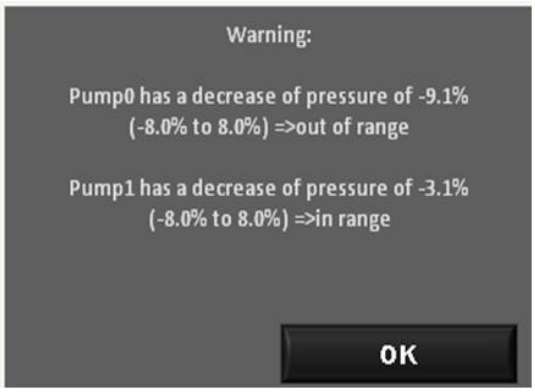
This button will open up an extra screen containing two tabs. The 'Edit Settings' tab is explained in detail in section 4.1.2.2 and can be used by the lab manager to create new accounts, change the location of the measurement database, import or export created formats and settings.

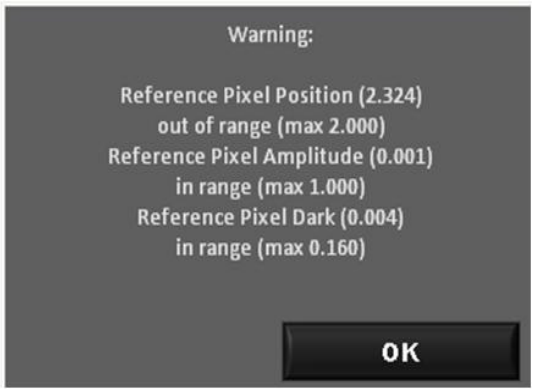
The 'System Info' tab contains a detailed description of the PC configuration (like free disc space and DropQuant version installed), detailed info on the attached DropSense96 (serial number, last calibration date, lamp and pump info), diagnostic checks and a ZIP function to save a general status of the Dropsense96 to be sent to the dealer for a first system analysis

6.2 Self testing

Upon instrument start-up or account log-in, the DropSense96 automatically performs diagnostic checks to ensure that it is functioning within specifications. This quick test is identical to the 'quick systems check' described in section 6.3.

When a part of the system is out of specification, then a warning screen will appear. Please contact your distributor for a recalibration of the DropSense96.


Warning:
Pump0 has a decrease of pressure of -9.1%
(-8.0% to 8.0%) =>out of range
Pump1 has a decrease of pressure of -3.1%
(-8.0% to 8.0%) =>in range
OK

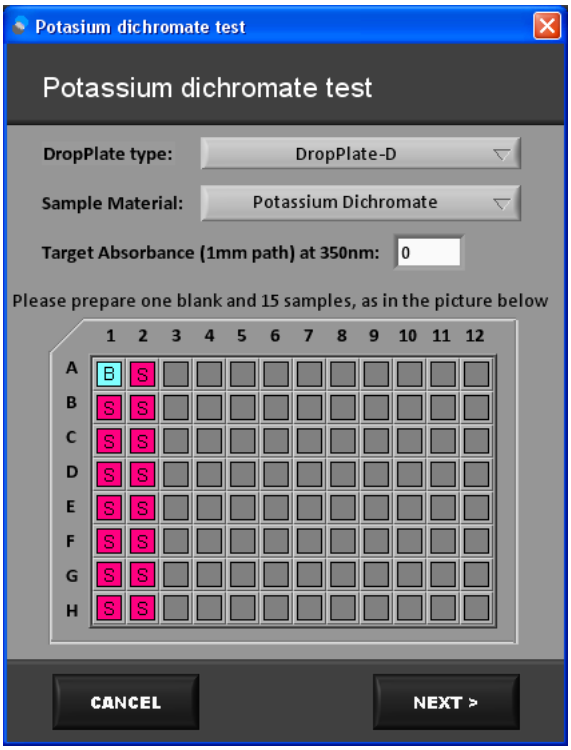


6.3 Potassium Dichromate test

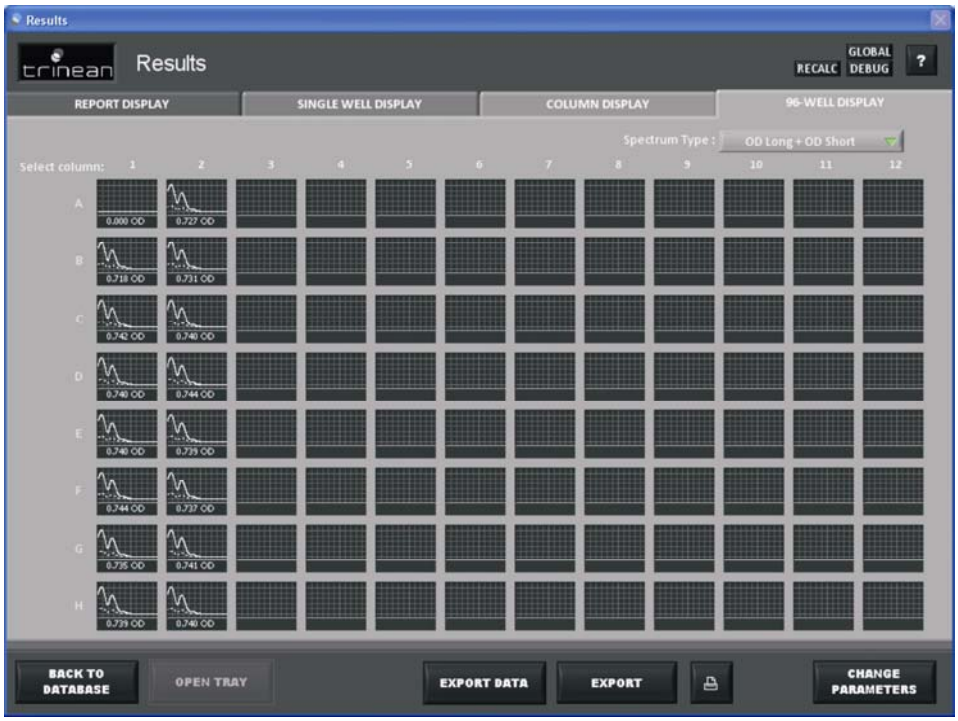
The DropQuant software includes a performance validation procedure as an easy check-up of the performances of the DropSense96. The check is based on the measurement of an aqueous potassium dichromate ($K_2Cr_2O_7$) solution with a verified concentration, commercially available from life science or spectroscopy companies. It is recommended to use a highly concentrated potassium dichromate solution (~0.4-0.8 OD at 350nm for a 1mm path length), which is better suited for calibration of the short path length measurements performed in the DropSense96. It is good practice to check the instrument's performance every four months with a fresh vial of $K_2Cr_2O_7$.

When pressing the 'Potassium Dichromate test' button, a new screen pops up with Potassium dichromate automatically entered as 'sample material'. Enter the DropPlate of choice (DropPlate-D or DropPlate-S) and the 'target absorbance' of the solution, given by the manufacturer, normalized for a 1mm path length. The DropQuant software will automatically calculate the expected OD values for all the path lengths used in the DropPlate. Load the blank and potassium dichromate solution on a DropPlate as indicated on the screen. It is preferred to use the potassium dichromate solvent as a blank. If not present, a pure water sample is a good alternative.

Then press the ‘Next’ button at the bottom of the screen to start the measurement.



When the calibration measurement is finished, the ‘96well display’ opens giving an overview. The ‘column display’ and ‘single well display’ can be used for detailed analysis of the spectra.



In the 'report display', the DropQuant software will show the results of the validation procedure. All OD values measured by the 4 optical systems in the DropSense96 are shown.

For every optical system, the mean of all measurements are calculated and compared with the expected OD value of the potassium dichromate solution. Color indications are foreseen giving a clear overview:

- **green color:** indicates that the measurement is within the expected measurement variation. The DropSense96 performs within specifications.
- **Red color:** indicates that the measurement does not fall within the expected variation. Contact your local distributor for a systems check-up.

A report (.txt, xls, .pdf).can be created using the 'export' button. This report can be stored on a disk for future reference.

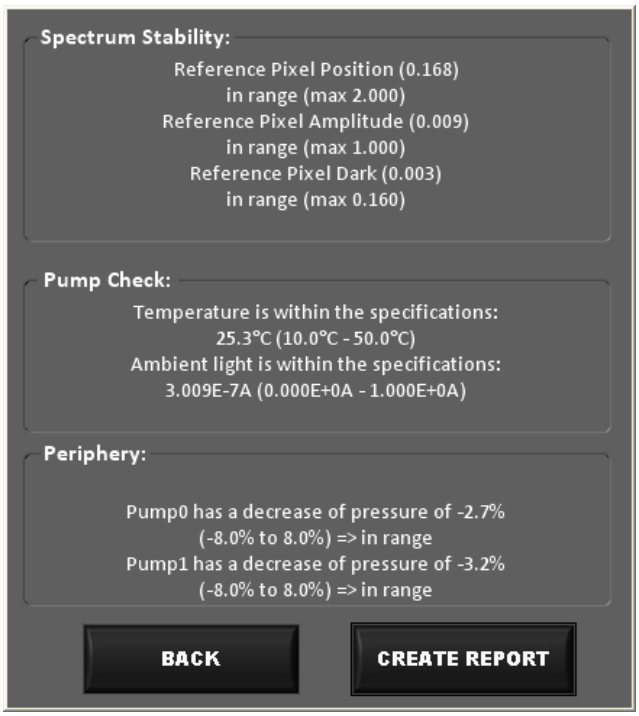
6.4 Quick system check

This tool performs a quick check of the spectrophotometer module and the pump module in the DropSense96. This quick check starts automatically when activated and takes about 30 seconds. The Quick system check consists of:

- Spectrophotometer check: the lamp spectrum is measured and compared to the lamp spectrum at calibration. The report summarizes the measured amplitude and position of the wavelength peaks, and gives a warning if these parameters deviate too much

from the calibration values. This indicates that the lamp or spectrophotometer may need service.

- Pump system check: the output pressure of the pumps is measured using the internal pressure sensors, and compared to the values at the calibration. This allows to check for degradation of the pumps.
- The operation of the internal background light sensor and the internal temperature sensor is checked.


Spectrum Stability:
Reference Pixel Position (0.168)
in range (max 2.000)
Reference Pixel Amplitude (0.009)
in range (max 1.000)
Reference Pixel Dark (0.003)
in range (max 0.160)
Pump Check:
Temperature is within the specifications:
25.3°C (10.0°C - 50.0°C)
Ambient light is within the specifications:
3.009E-7A (0.000E+0A - 1.000E+0A)
Periphery:
Pump0 has a decrease of pressure of -2.7%
(-8.0% to 8.0%) => in range
Pump1 has a decrease of pressure of -3.2%
(-8.0% to 8.0%) => in range
BACK
CREATE REPORT

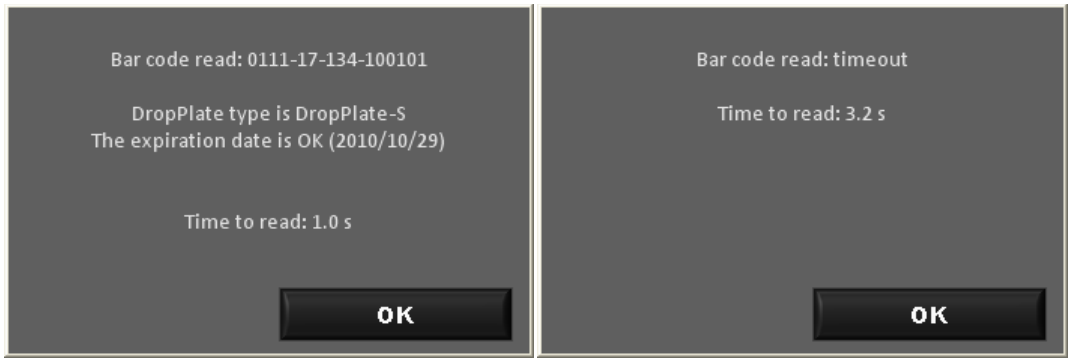
After the check, a pop-up window shows the most important results. An Excel report can be created using the 'create report' button. This report can be stored on a disk for future reference.

It is designed is a fast check so not all system parameters are checked. The most important parameter that is not checked is the accuracy of the DropPlate positioning. The Potassium Dichromate test is a better check of the positioning accuracy.

6.5 Check barcode reader (optional accessory)

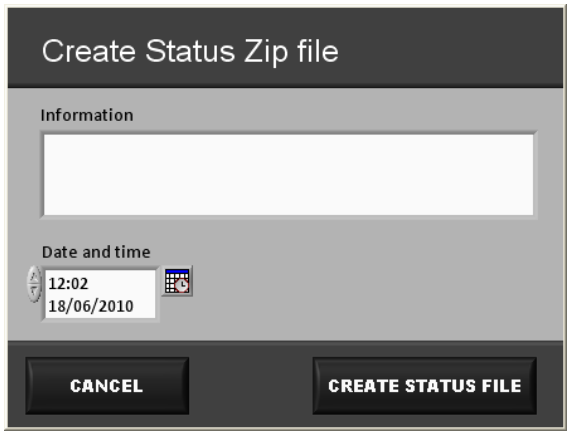
The functionality of the internal barcode scanner can be tested using the "check barcode reader" button: simply push this button and the barcode of the DropPlate in the instrument will be read and shown. If there is no internal barcode scanner installed on the DropSense, this option will not be active and displayed in grey.

Use the 'Open Tray' button to load a DropPlate with barcode in the system before starting the barcode check function. The conversion time should be under 2 seconds. If the scanner cannot read a code in 3 seconds, then a time-out warning is given. In that case, check the quality of the barcode label and try again. If the time-out warning reappears, then contact the dealer for service.



6.6 Generate status ZIP file

This function creates a ZIP file with copy of the internal log files and other information. Use this function in case of problems. This ZIP file can be send to the dealer for further investigation.



6.7 Nucleic acid controls

Several nucleic acid products with an accurate DNA/RNA concentration are commercially available. These products can be used as routine laboratory control solutions to check the reproducibility and accuracy of the DropSense96. These controls are valid to use with properly calibrated DropSense96 instruments. The obtained spectra can be used as an example of what high quality nucleic acid spectra look like and what the expected A260/A280 ratio is of pure nucleic acid samples.